ECHA
EUROPEAN CHEMICALS AGENCY

DRAFT: Guidance on the assessment of risks to bees from the use of biocides

April 2023

Disclaimer

This document aims to assist users in complying with their obligations under the Biocides Regulation. However, users are reminded that the text of the Biocides Regulation is the only authentic legal reference and that the information in this document does not constitute legal advice. Usage of the information remains under the sole responsibility of the user. The European Chemicals Agency does not accept any liability with regard to the use that may be made of the information contained in this document.

Version	Changes	

Guidance on the assessment of risks to bees from the use of biocides
Reference: xxxx-xxxx-xxxx
ISBN: xxxx-xxxx-xxxx
Cat. Number: ED-xxxxxx-xxxx-xxxx
DOI: xx.xxxx/xxxxxx
Publ.date: Month 20xx
Language: EN

If you have questions or comments in relation to this document, please send them (quote the reference and issue date) using the information request form. The information request form can be accessed via the Contact ECHA page at:
http://echa.europa.eu/contact

European Chemicals Agency

P.O. Box 400, FI-00121 Helsinki, Finland

Abstract

The European Commission asked ECHA to develop a guidance for assessing the risks to arthropod pollinators (including bees) from the use of biocides, taking into account EFSA's Guidance on the risk assessment of plant protection products on bees. This Guidance Document describes how to perform a risk assessment for bees, in accordance with Article 19(1)(b)(iv) of the BPR. It proposes a tiered approach scheme for biocidal active substances for the exposure estimation in different scenarios, hazard characterisation and a risk assessment methodology covering both dietary and contact exposure. This document also provides recommendations for higher tier assessment, metabolite risk assessment and biocidal product risk assessment (mixtures). For arthropod pollinators other than bees, an overview of the literature and a database search on the ecology and sensitivity of non-bee pollinators are provided together with recommendations for further research and considerations for future development of guidance.

Keywords

Bees, pollinators, biocides, Guidance Document, *Apis mellifera*, *Bombus spp*., solitary bees, risk assessment, lower tier studies, Specific Protection Goals, toxicity, exposure

Summary

In December 2019, ECHA received a mandate from the European Commission to develop a guidance for assessing the risk to pollinators (including bees) from the exposure to biocides. According to the mandate, the ECHA should take into account the revised EFSA Guidance document on the risk assessment of plant protection products on bees, which was released in [May 2023].

The mandate also stated that the ECHA Guidance on pollinators should specify the information required to enable a conclusion by the evaluating authority on whether the biocidal product complies with the criteria under Article 19(1)(b)(iv) concerning bees and other arthropod pollinators. However, due to the current lack of data on non-bee pollinators, this guidance document only covers the risk assessment to bees from the use of biocides.

ECHA has developed this guidance document together with a group of experts from different Member States, as well as with the support from stakeholder organizations.

This guidance document provides a methodology to assess the risk to honey bees that are exposed to biocides. This is done by following a tiered approach for the exposure and the effect assessment. In the risk assessment of honey bees, the *magnitude* dimension of the Specific Protection Goals (SPGs) is applied as a threshold for acceptable effects. In regard to bumble bees and solitary bees, a risk assessment methodology is provided but the magnitude dimension of the SPG is not defined due to current lack of knowledge.

This guidance considers two main routes of exposure to bees, via intake of contaminated pollen and nectar through the diet, and via contact, when the bees come to physical contact with the biocidal product.

In the risk assessment, a tiered approach is applied both for exposure and effect assessments, i.e., an exposure-Tier and an effect-Tier have been defined. In the exposure tiers, residue intake or residue deposition need to be quantified by calculating the Predicted Exposure Quantity (PEQ) to address the dietary and the contact exposure of the bees from the use of a biocide through

the different routes of exposure. In the effect tiers, the imposed exposure is called 'Dose' in the laboratory tests or 'Estimated Exposure Dose' in the higher tier tests.

The routes of exposure to bees for both exposure and effect assessments are approached considering both acute and chronic effects, and adults and larvae as different life-stages. Thereby, four risk cases have been defined: acute-contact; acute-dietary; chronic-dietary; larvae-dietary. For each of these risk cases, a PEQ_j is derived in the exposure estimation with suffix j indicating the specific risk case.

The Guidance proposes a risk assessment approach to assess the risk to bees from product type (PT) 18 (insecticides, acaricides and products to control other arthropods) emission scenarios. The possible sources of exposure covered in the guidance are (1) overspray, (2) application of manure/sludge to agricultural soil or grassland, (3) small scale spray around the house, and (4) irrigation of private gardens with treated water.

In the effects assessment, the lower tier assessment will define dose response curves (DRC), which are parameters to describe the steepness of the dose-response relationship obtained from the standard laboratory test.

As part of the effect assessment, this guidance document includes two additional aspects for honey bees: considering and assessing whether the concerned compound presents increasing toxic effects due to long-term exposure to low doses – Time Reinforced Toxicity (TRT) and potential concerns due to sublethal effects.

The guidance also provides advice for higher tier effect assessment as a potential way for refinement, in case unacceptable effects are observed in the lower tier assessment. Furthermore, a risk assessment scheme for metabolites and biocidal products (mixtures), and considerations of risk mitigation measures, instructions for use and warning sentence are included in the document.

This guidance document for the risk assessment of bees from the use of biocides is developed by taking into account the existing guidance available by EFSA for the risk assessment of plant protection products. For further information on the detailed aspect of the risk assessment methodology as well as the scientific background information, the reader is referred to the EFSA guidance (Revised guidance on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees)) and its supplementary documents.

Table of Contents

Table of Figures

Table of Tables

1. Introduction

1.1. Background and Terms of Reference as provided by the European Commission

In December 2019, the European Commission ("COM") mandated ECHA to develop a guidance for assessing the risks to arthropod pollinators (including bees) from biocides exposure to ensure a high and harmonised level of protection of the environment, taking into account the *Revised guidance on the risk assessment of plant protection products on bees (Apis mellifera, Bombus spp. and solitary bees)* by EFSA ("**EFSA Bee guidance**"). In addition, ECHA was requested to specify the information required to enable a conclusion by the evaluating authority on whether products comply with the criteria under Article 19(1)(b)(iv) of the Biocidal Products Regulation (BPR) concerning bees and other arthropod pollinators.

According to the mandate, the following elements were to be considered by ECHA when addressing this question:

- *In order to develop a specific guidance to assess the risk to arthropod pollinators (including bees) from the use of biocides, ECHA shall use any information already available, and in particular the past and current work of EFSA in this field.*

- *To ensure that all available information can be considered in the opinion a targeted consultation of stakeholders should occur. For this consultation, if ECHA considers it appropriate, an overview of biocidal active substances and biocidal products to which arthropod pollinators could be exposed and may trigger directly or indirectly the occurrence of adverse effects in them could be prepared.*

- *The current references to the assessment of risk to arthropod pollinators included in the ECHA Guidance on the Biocidal Products Regulation shall also be considered, along with the work in this field already carried out by the competent authorities and scientific bodies from the EU Member States*

Throughout the development process of this ECHA Guidance on the assessment of risks to bees from the use of biocides ("**ECHA Bee guidance"**), ECHA carried out several actions to consider the elements included in the mandate:

- Establishment of Expert Group ("**ECHA EG**"): A scientific expert group composed of experts from Member States with specific scientific competence in risk assessment to bees, other arthropod pollinators and bee biology with the support from experts from the European Food and Safety Authority (EFSA) was set up by ECHA.

- Consideration of EFSA Bee guidance: In reference to the work being done at EFSA, ECHA and EFSA were in constant communication in relation to risk assessment to bees. The EFSA Bee guidance published in [May 2023] has been taken as reference in the development of this ECHA guidance document for assessing the risks to bees from the use of biocides.

- Consultation of stakeholders: A dedicated Guidance Consultation Expert Group (GCEG) was established by ECHA for the consultation of stakeholders. The group was defined with a specific role, composition and responsibility. Written consultation and dedicated meetings were organised to consult BPC Environment Working Group, Biocidal Products Committee and the representatives of Members States Competent Authorities for the implementation of Regulation EU) No 528/2012.

- Scoping document: In the initial stage of the guidance development, the ECHA EG chose to start with a scoping document before proceeding to the actual drafting phase of the ECHA Bee guidance. The scoping step was critically important in the guidance development for biocides, as there was no specific guidance available to assess risk for bees or other arthropod pollinators. Results of the work have been reported in a standalone document (ECHA 2020).

- Risks to arthropod pollinators other than bees (**non-bee pollinators, NBP**): Within the ECHA EG, several experts focussed on NBPs with the goal of ensuring that a risk assessment methodology will be available in the future to protect these organisms. Firstly, a literature review related to the ecology and the sensitivity to insecticides of Diptera, Lepidoptera, non-bee Hymenoptera, and Coleoptera was done. Furthermore, a collection of toxicity endpoints of NBPs exposed to active substances was conducted. The results of the work have been reported in a standalone document (ECHA 2022).

In reference to the mandate, the present guidance document is intended to assist applicants and competent authorities to carry out **assessment of risks to bees** from the use of biocide active substances and biocidal products.

The following areas are covered in this guidance document:

- Introduction (Chapter 1)
- Scope of the Guidance Document (Chapter 2)
- Overview of the risk assessment (Chapter 3)
- Problem formulation (relevant exposure scenarios) (Chapter 4)
- Exposure assessment methodology (Chapter 5)
- Effect assessment in lower tiers (Chapter 6)
- Lower tier risk assessment (Chapter 7)
- Time reinforced toxicity and sub-lethal effects (Chapter 8 and 9)
- Higher tier risk assessment (Chapter 10)
- Metabolite assessment (Chapter 11)
- Mixtures (Biocidal products) (Chapter 12)
- Risk mitigation measures, instructions for use, and warning sentence (Chapter 13)
- Recommendations (Chapter 15)

In addition, the approach for the development of guidance for arthropod pollinators other than bees (NBPs) is explained (Section 1.6).

1.2. Legal framework

Regulation (EU) No 528/2012 of the European Parliament and of the Council (Biocidal Products Regulation, the BPR) lays down rules and procedures for approval of active substances in biocidal products and for the authorisation of biocidal products.

The process of evaluation of active substance applications is given in Article 8 (BPR) and the common principles for the evaluation of dossiers for biocidal products (including the representative biocidal product in the context of active substance approval) is given in Annex VI (BPR). The evaluating or receiving competent authority (CA) uses the data submitted in support of an application for active substance approval, or authorisation of a biocidal product, to make a risk assessment based on the proposed use of the (representative) biocidal product.

Article 19(1)(b)(iv) of the BPR establishes that a biocidal product, when used as authorised, shall not generate unacceptable effects on the environment, having particular regard to the impact of the biocidal product on non-target organisms, which, among many other organisms, include also bees and other arthropod pollinators under the terrestrial compartment. The risk assessment is therefore a principal part of the evaluation process.

Study data and other information must enable the conduct of a proper risk assessment in order to allow a decision on the suitability of the substance to be approved or the product to be authorised. The BPR sets out rules on information requirements that are specified for active substances in Annex II, and for the respective biocidal products in Annex III. The common core data set (CDS) forms the basis of the requirements and is information that always has to be submitted. The additional data set (ADS) includes supplementary information that may be required depending on the characteristics of the active substance and/or the product-type and on the expected exposure of humans, animals, and the environment. Data requirements in relation to risk assessment of bees are explained in section 6.1.2.

While the COM mandate to ECHA includes a requirement to take into account the EFSA Bee guidance, it is important to notice that there are differences in the assessment of plant protection products (PPPs) and biocidal products (BP). For instance, the type of application of biocides is fundamentally different to the type of application of PPPs which leads to potentially different routes and levels of exposure of arthropod pollinators to active substances which has an impact on the focus of the ECHA Bee guidance. In addition, at the time of the ECHA Bee guidance development, there was a difference in the amount of available data for arthropod pollinators in pesticide and biocide dossiers, especially from experiments with applications comparable to biocides exposure patterns. Furthermore, while in the EFSA Bee guidance the scope is limited to the species *Apis mellifera*, the family *Bombus spp*. and the various groups of solitary bees, the ECHA Bee guidance, in line with the COM mandate, in addition considers the first steps needed for the development of guidance on assessment of risks to other arthropod pollinators.

In regard to the 'unacceptable effects on the environment', the aim of the BPR is to provide sufficient protection to the environment from exposure to biocidal active substances and biocidal products at a general level. That is normally, for aquatic, terrestrial, and sewage treatment plant (STP) compartment, performed by comparing the predicted environmental concentration (PEC) in the relevant environmental compartment with the predicted no effect concentration (PNEC) below which no adverse effects in the environmental compartment are expected to occur (PEC/PNEC ratio). In the context of PPPs, unacceptable effects on the environment, including non-target species and impact on biodiversity and the ecosystems, are among the approval criteria. EFSA PPR Panel (2010) and EFSA Scientific Committee (2016) have proposed a methodology to define specific protection goals (SPGs) based on ecosystem services and biodiversity with the underlying principle that the general protection goal of Plant Protection Products Regulation (PPPR) may be achieved via the protection of providers of ecosystem services.

1.3. Specific Protection Goals

The environmental protection goals outlined in BPR encompass biodiversity and the ecosystem. These broad goals are translated into specific (operational) protection goals (SPGs), or threshold of acceptable effects on colony/population size, in order to be directly applicable for bee risk assessment in line with the methodology defined in the EFSA Bee guidance. An overview of the SPGs for honey bees, bumble bees, and solitary bees from the EFSA Bee guidance is presented

in Table 1.

Table 1: Overview of the SPGs for honey bees, bumble bees, solitary bees from EFSA Bee guidance.

Dimensions	*Honey bees*	*Bumble bees*	*Solitary bees*
Ecological entities	*Colony*	*Colony*	*Population*
Attribute	*Colony strength*[1]	*Colony strength*[1]	*Population abundance*
Magnitude[2]	*≤ 10%*	*Undefined*	*Undefined*
Temporal scale	*Any time*	*Undefined*	*Undefined*
Spatial scale	*Edge of field*	*Edge of field*	*Edge of field*

At the 93rd meeting of representatives of Members States Competent Authorities for the implementation of Regulation (EU) No 528/2012 concerning the making available on the market and use of biocidal products[3], the Commission indicated, underlining the differences between biocides and plant protection products, that these goals should be considered as far as possible in the development of ECHA Bee guidance.

The following definitions of the dimensions are presented in the EFSA Bee guidance.

*The **ecological entity** dimension refers to the level of biological organisation for the identified service providing units, i.e., populations that deliver a given ecosystem service.*

*The **attribute** dimension allows identify the most ecologically relevant elements that must be protected relative to the ecological entities.*

*The **magnitude** dimension refers to the level of tolerated effects for the attribute to be measured relative to the defined ecological entities.* Note that for the *EFSA guidance, risk managers agreed on a magnitude dimension for honey bees (A. mellifera) for the entire EU corresponding to a value of 10% as the maximum permitted level of colony size reduction following pesticide exposure. For bumble bees and solitary bees, based on the consolidated information provided in EFSA et al. (2022a), an evidence-based decision for a threshold of acceptable effects could not be finalised by risk managers due to the lack of data. The majority decision was for an 'undefined threshold' that was given as an option in EFSA et al. (2022a).*

*The **temporal scale** dimension defines the duration of tolerated effects.*

*The **spatial scale** dimension 'edge of field' refers to the location of the colonies/populations, i.e., directly adjacent to the treated field, from where the bees forage in the treated field or immediate off-field areas.* For biocides, this dimension refers to the location of the colonies/populations directly next to the treated area.

1.4. Pathways of biocides exposure for bees

Biocidal products are used to protect humans, animals, materials, or articles against harmful organisms like pests or bacteria, by the action of the active substances contained in the biocidal product. Biocides are widely used and there is concern that emissions of biocides in the environment may result in the exposure of bees. The way a biocide can become a source of exposure for bees is determined by the emission of a biocide to the environment. Bees may

[1] Colony strength is defined operationally as colony size reduction.
[2] The magnitude was the only dimension reviewed and agreed by EFSA risk managers for the EFSA guidance. For bumble bees and solitary bees, a threshold will be defined when more data will become available.
[3]Minutes of the 93rd meeting of representatives of Members States Competent Authorities for the implementation of Regulation (EU) No 528/2012 concerning the making available on the market and use of biocidal products, available at https://health.ec.europa.eu/events/93rd-meeting-expert-group-implementation-biocidal-products-regulation_en

come into direct contact with biocides (e.g., droplets of spray), as well as be exposed via contaminated matrices (e.g., by contact with contaminated surfaces or the consumption of nectar or pollen).

An overview of the pathways of exposure for bees to biocides which are evaluated in this guidance is shown in Figure 1 (adapted from EFSA Bee guidance).

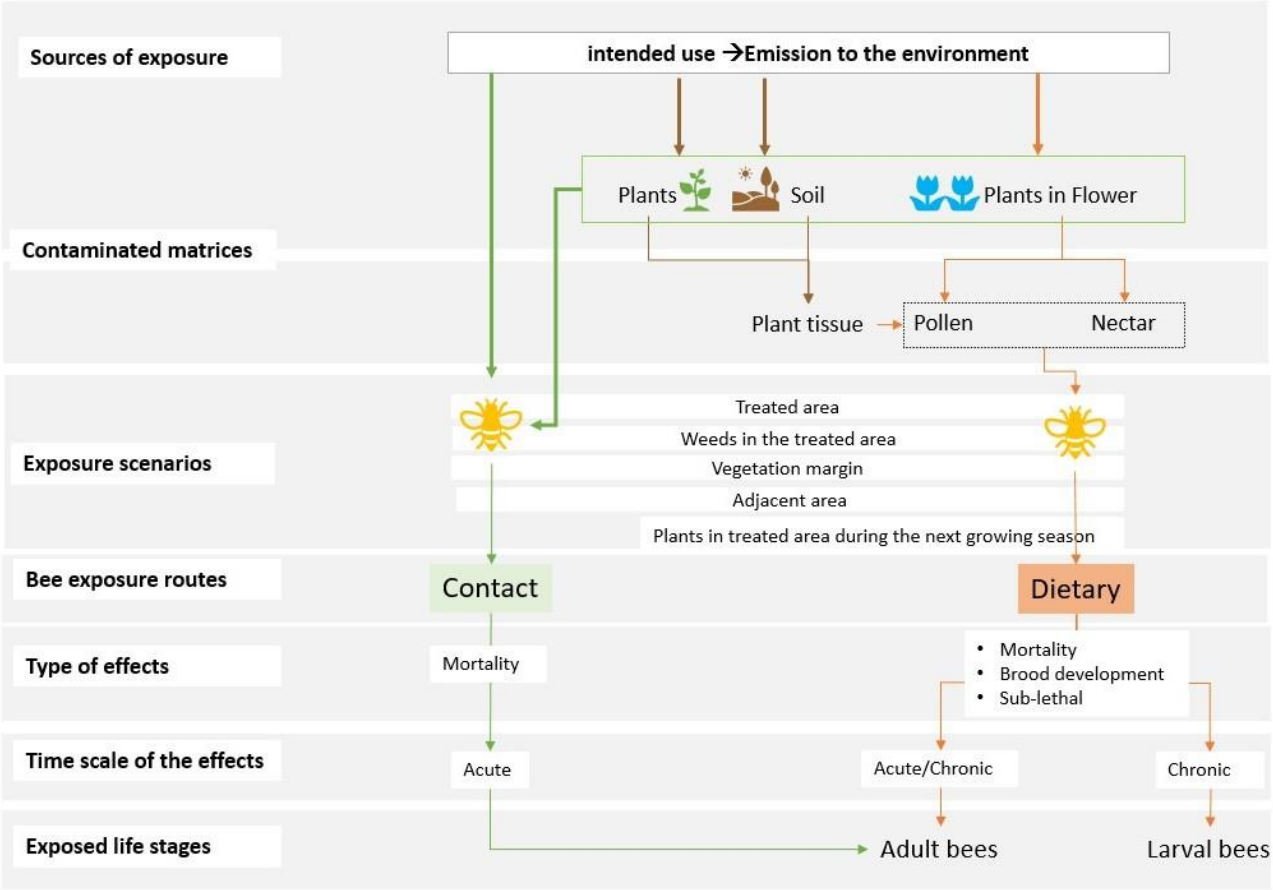


Figure 1: Bee exposure pathways evaluated in the context of biocide risk assessment (adapted from EFSA Bee guidance)

The following series of processes of the way bee matrices may be contaminated is adapted from the EFSA Bee guidance: *The bee matrices may be contaminated directly (e.g. by spray liquid or dust deposits to the pollen/nectar) or via a series of processes: For example, 1) a PPP (or biocide) is sprayed onto the plant surface (e.g. leaves) → the PPP (or biocide) enters the plant and is distributed through the plant tissue → reaches the reproductive organ(s) → excreted into e.g. pollen and nectar; 2) a proportion of the PPP (or biocide) is sprayed onto the soil →a proportion of the PPP (or biocide) is taken up by the roots of the plant → distributes within the plant → reaches the reproductive organ(s) → diffused to e.g. pollen and nectar.*

Bees may be exposed to contaminated matrices either in areas where biocides are used or in areas that have been unintentionally contaminated. In order to aid in the development of the exposure assessment, various exposure scenarios for the assessment of risk to bees due to the use of biocides have been defined. These biocide exposure scenarios have been developed on the basis of the exposure scenarios presented in the EFSA Bee guidance. The transposition of the scenarios and the correspondence of terms under biocides and PPPs is presented in Table 2.

Table 2: Exposure scenarios covered in the scope of the EFSA Bee guidance (PPP) and ECHA Bee guidance (Biocides).

EFSA (PPP)	ECHA (Biocides)
Treated crop	Treated area
Weeds in the field	Weeds in the treated area
Plants in the field margin	Vegetation margin
Adjacent crop	Adjacent area
Succeeding crop	Plants in treated area during the next growing season

It is assumed that bees may forage on a plant if it is attractive to bees. In the treated area scenario, it is therefore assumed that the plant is flowering. Similarly, weeds growing in the same area are assumed to be flowering at the time of application of the biocidal product.

Furthermore, the ECHA Bee guidance considers that, for spray applications, spray drift during spray application reaches areas beyond the edge of the treated area and therefore bees could be exposed by foraging on plants growing in a vegetation margin or adjacent area. Spray drift deposition is assumed to decrease with the distance from the treated area. For both the vegetation margin and the adjacent area scenario it is assumed that there are flowering plants at the time of application, and that all foragers from a hive forage on the attractive plant as a worst-case.

The ECHA Bee guidance presumes that bees may forage also on plants in the area during the next growing season. This scenario takes into account that soil residues of substances may lead to root uptake in the next growing season or the following year and that these residues are subsequently transported via the plants to nectar and pollen.

The relevance of the exposure in those scenarios as well as the level of exposure varies pending on the source of exposure (see Chapter 4 and Chapter 5).

There are two main ways (i.e., bee exposure *routes) through which a PPP (or biocides) or their residues can reach the bees (different life stages) in the above defined scenarios and potentially causing adverse effects:*

- *By contact: it occurs when bees enter in physical contact with the PPPs (or biocides) or with contaminated matrices, but that does not involve ingestion.*
- *By dietary: it occurs when bees orally consume contaminated material and therefore, they ingest residues of PPPs (or biocides) with their diet.*

In addition, the following assumptions from the EFSA Bee guidance have been taken over for the ECHA Bee guidance:

- Insufficient information is available to consider the exposure through inhalation
- It is acknowledged that for most species of bumble bees and solitary bees nesting in the soil, repeated exposure by contact with contaminated soil/mud/leaves may be relevant. However insufficient information is still currently available to address these exposures.
- EFSA has evaluated the relevance of exposure via consumption of contaminated water and concluded that data were not sufficient to achieve a reliable estimation of quantified water consumption or frequency and magnitude of water collection. In consideration of this, exposure from contaminated water is not included in the ECHA Bee guidance document.
- In the EFSA Bee guidance, dietary exposure is considered as ingestion of contaminated nectar and pollen for both adult bees and larvae that could cause lethal or sublethal

effects on acute and chronic basis. Other contaminated matrices (e.g., honey dew, extrafloral nectaries, resin, wax etc.) that could lead to oral residue intake are not explicitly covered in this guidance due to lack of sufficient data to propose a quantitative risk assessment approach.

Overall, areas which are not covered in the ECHA Bee guidance may be addressed in future revisions of the guidance document and the ECHA EG recognises the need to generate further research and data. ECHA Bee guidance Chapter 15 outlines areas where further research is needed.

1.5. Bee ecology

Pollinating arthropods can be divided in two main groups: "bees" and "non-bee pollinators" (NBPs). The group "bees" (taxonomic order: Hymenoptera, taxonomic family: Apidae) covers honey bees (*Apis mellifera*), bumble bees (*Bombus sp.*), as well as solitary bees (e.g., *Osmia spp., Megachile sp., Andrena sp.*). For more information on bee ecology, please refer to the Supplementary Document of the EFSA Bee guidance (Section 1.1) which describes general information on bee life history as well as specific aspects of honey bees, bumble bees and solitary bees.

The other group, the NBPs, is much more diverse and consists of different taxonomic orders. The most important NBPs are flies (Diptera), butterflies and moths (Lepidoptera) and beetles (Coleoptera). Also, wasps (Vespidae), belonging to the taxonomic order "Hymenoptera", are important pollinators. For more information on NBPs, please see Chapter 1.6.

1.6. Non-bee pollinators

According to the Commission's mandate (see section 1.1), guidance for the risk assessment from the use of biocides was requested not only for the species *Apis mellifera*, the family *Bombus spp*. and the various groups of solitary bees (bee pollinators), but also for other arthropod pollinators.

The first version of the ECHA Bee guidance will consider other non-bee pollinators (NBPs) to a limited extent only. The reason behind this decision is the lack of information in the literature regarding inter-species sensitivity to biocides and lack of standardised test guidelines for NBPs. This is currently preventing the development of a scientifically based methodology to assess the risk arising from the use of biocides to these non-bee taxa. Nevertheless, as a part of the work under the COM mandate and guidance development, the ECHA EG performed a literature and a database search (ECHA 2022) to assess the available data to investigate the sensitivity of *Diptera*, *Lepidoptera*, non-bee *Hymenoptera*, and *Coleoptera* to insecticides and to compare it to that of honey bees. The aim of this analysis was to find out whether the honey bee could be used as a surrogate species when assessing risks to NBPs.

The ECHA EG's scientific report (ECHA 2022) explains which arthropod species may be regarded as relevant pollinators, then further describes the main characteristics of relevant NBP orders, their ecological profiles, and roles as pollinators. The report also outlines the variations in life stages (e.g., foliage- or soil-dwelling) and feeding habits (e.g., herbivorous or feeding on pollen/nectar). As the data available were few and the distribution of data were uneven across the active substances and NBP taxa considered, a comparison of sensitivity data between bee and non-bee species was only possible for some representatives of the orders *Lepidoptera* and *Coleoptera*, and three *Dipteran* species. Thus, the report found out that at this time it is not possible to conclude on sensitivity differences between bee and NBPs, as information is scarce for all relevant taxa. In summary, the report identifies the following data gaps:

- Lack of validated standard test guidelines for NBPs
- Lack of information on the basic biology, ecology and e.g., feeding behaviour to allow

conclusion on species vulnerability and further the selection of representative species (surrogates) or alternatively assessment factors for NBPs
- Lack of information on the most relevant route of exposure, and at which life stage NBPs are most exposed to biocides
- Lack of commercially available NBP species

In the report, ECHA EG has highlighted that also NBPs significantly contribute to pollination. NBPs can be exposed to biocidal products during application (contact), via soil (contact), and/or by uptake of contaminated matrix (oral). Therefore, NBPs should be considered in the risk assessment for biocidal products.

The full details on the sensitivity analysis, results and conclusions are explained in the ECHA 2022 publication.

Regarding the risk assessment for NBPs, the ECHA EG concluded that future development of guidance is needed. Furthermore, the ECHA EG has agreed to consider the NBPs under the terrestrial compartment of the environmental risk assessment (according to BPR Annex VI)[4].

2. Scope of the Guidance Document

This document is intended to provide guidance to applicants and risk assessors for the risk assessment of bees in the context of the evaluation of biocidal products and their active substances under Regulation (EC) No 528/2012 for authorisation process at EU or Member State level and the approval at EU level, respectively.

The ECHA Bee guidance covers the risk assessment for chemical biocidal substances, applied as or reaching the environment through outdoor spraying, manure/sludge application, and irrigation. The ECHA Bee guidance covers mainly these sources of exposure, for which exposure estimation approaches are available and consolidated, although the principles of proposed risk assessment schemes may be relevant for other sources of exposure as well. The ECHA Bee guidance does not cover the risk assessment for micro-organism active substances. According to the EFSA Bee guidance, micro-organisms are not covered by the guidance since specific considerations are needed. According to EFSA Bee guidance, for indoor uses, no exposure to bees living in the surrounding areas is expected and therefore a risk assessment is normally not necessary. However, it is noted in the EFSA Bee guidance that for applications made indoors and where seedlings are subsequently transported to the field, exposure to bees may occur. By analogy to this, for biocides that are applied in animal housing and where treated manure/sludge is subsequently transported to the field, exposure to bees may occur.

When a biocide is used in a way that is likely to result in significant exposure of bees that is not covered by the ECHA Bee guidance, it is considered that the applicant has the responsibility to provide a proper characterisation of the exposure in line with the principles of this guidance.

2.1. Focus on PT18 active substances and products

2.1.1. Emission scenarios with potential exposure to bees

The strategy used to identify emissions of biocides in the environment, with potential exposure to bees significant enough to be further considered for exposure assessment, is outlined below.

[4] Currently there is no method available for biocides on how to perform the risk assessment for non-target arthropods, thus an update of BPR Guidance Volume IV: Environment Part A: Information Requirements and Volume IV Environment - Assessment and Evaluation (Parts B + C) is foreseen once new data on NBPs become available.

The ECHA EG screened and evaluated all available Emission Scenario Documents (ESDs) and Technical Agreements for Biocides (TAB) for the 22 biocide product types (PTs). The intention was to rule out applications of biocides where exposure to bees is not likely. The potential for exposure to bees was considered from releases, i.e., emissions to the first receiving compartments, and emissions to subsequent compartments separately. Manure is, for example, not considered as an "environmental compartment" as such, and for scenarios where manure is applied on soil, soil is considered as a first receiving environmental compartment.

The following criteria were considered relevant in order to identify biocides emission scenarios which might potentially contribute to the exposure of bees following release:

- outdoor use/release
- release pathway/application type considered relevant (e.g., spray drift)
- release scale of a certain magnitude (e.g., spray or manure applications)
- insecticidal mode of action

Additional considerations were made in some cases with regard to the release being temporary, or whether bees are likely to be present. Expert judgements were made to some scenarios where exposure to bees was not considered probable, due to e.g., the compartment not being relevant for bees, or area not attractive or not of interest to bees due to lack of food sources. On the basis of the work of the ECHA EG and input from Accredited Stakeholder Organisations (ASO) and Member State consultations, it was concluded that biocide emissions from insecticides, acaricides and products to control other arthropods (PT18) have potential exposure to bees significant enough to warrant exposure assessment. The ECHA Bee guidance therefore proposes a risk assessment approach to assess the risk to bees for the PT 18 Emission Scenarios presented in Table 3. Note, however, that this list is not exhaustive and does not exclude future scenarios which might become relevant.

Table 3: PT 18 ESD Scenarios with potential exposure to bees meriting exposure assessment

PT 18 ESD Scenario	Potential Exposure to bees
Outdoor applications (spray application) *, ***	YES
Insecticides application in animal housings and at manure storage systems**	YES
Outdoor large-scale spraying (TAB ENV 248)	YES
Irrigation scenario (TAB ENV 205)	YES

*Emission Scenario Document for Insecticides, Acaricides and products to control other arthropods for household and professional uses
** Emission Scenario Document for Insecticides for Stables and Manure Storage Systems
***Note that wasp and hornets spray are not considered in this guidance.

2.1.2. Bait and spot applications and nest spray application

PT18 products applied as bait or spot applications could be broadly defined as products in the form of gel, blocks, liquids, granule, or powder, containing both food attractants and an insecticide active substance. The ECHA EG initially considered that bees may forage directly on sugar-containing bait products. However, literature research and ASO consultations showed that there is no data that confirms this assumption. During an ASO consultation conducted in 2022, several peer-reviewed publications were submitted which indicate that the attractiveness of bait products is low for insect pollinators in general and for bees in particular. Firstly, the publications showed that baits in solid form are not attractive to bees because the bees prefer to access sugar in liquid form. This is because bees have mouthparts that are used to lap nectar, to ingest it by dipping their tongue into and then extracting it from the nectar (Kim et al., 2011). Lapping is thus not possible for solid baits and also limited for high viscosity baits. In this regard, Nicolson (2013) also showed that honeybees prefer to forage on less viscous nectar. Secondly, studies have shown that as long as food alternatives such as honey, nectar or pollen were available,

bees prefer to move to these sources instead of sugar-containing baits (Toledo-Hernández et al., 2021; Mangan et al., 2009)

Furthermore, for such local spot and bait applications, a major effect on wild bees leading to colony collapse at a larger scale seems unlikely to occur. In conclusion, based on the available information, bee exposure through direct consumption of PT18 bait products is not considered in this guidance.

For the outdoor treatment of wasp or hornet nests, no quantitative risk assessment is required because exposure to bees is considered to be very limited compared to the other sources of exposure presented in this ECHA Bee guidance (see Chapter 5). The size of the exposed soil area from deposition after the spray application (30 % of the biocide applied) onto the nests is only a small circular surface of 50 cm diameter just below the treated nest (ESD PT18, 2008). No spray drift beyond this area is assumed. It is very unlikely that a significant effect on the colony size of bees is expected from this use.

The same reasons also apply for spot applications with e.g., baits (including application around terraces). The size of the exposed soil area from emissions during spot application is 0.25 m^2 (ESD PT18, 2008) or 8.5 m^2 for applications on terraces (TAB entry 154, TAB October 2022). Example calculations were conducted by the ECHA EG with a hypothetical toxic active substance and those calculations confirmed that with a Tier 2 assessment (refinement of exposure with PECpw,2, see Chapter 5.3), such a biocidal use will always result in acceptable risk in regard to exposure to bees.

2.2. Comparison between EFSA and ECHA Bee guidance documents including justifications of all uses and substances not considered in the ECHA Bee guidance

Although the EFSA Bee guidance exclusively refers to agricultural setting, there is a potential to transfer the logic of the PPP exposure scenarios to biocides uses. It is assumed that for the biocides uses, which entail applications by spray, spreading of manure or sludge containing biocides on agricultural fields or grassland, as well as irrigation, the exposure scenarios in the EFSA Bee guidance may be taken over for biocides by applying certain adaptations. For some biocides uses, which are outside of a field context, some caution needs to be applied when applying the principles of the EFSA Bee guidance.

It is important to highlight the differences in the assessment of PPPs and biocidal products that result in the approach in the ECHA Bee guidance being different in some aspects to the EFSA Bee guidance. In consideration of how to make best use of the available methodology of EFSA Bee guidance, the following has been agreed with regards to differences and similarities between the assessment framework for PPPs and biocides:

- The standard testing methods for assessing the risk to *Apis mellifera*, *Bombus* spp. and solitary bees can be generally used in the ECHA Bee guidance.

- Some exposure calculations for biocidal uses can follow representative exposure scenarios considered in the EFSA Bee guidance, with certain adaptations. Adaptations are needed as the application methods and use context as well as the availability of certain basic information for PPP and biocides are not the same (e.g., application to crops/fields versus application around houses, type of treated plants, lack of measured residue levels, etc.).

- The risk assessment principles presented in the EFSA Bee guidance at the first tiers can in principle be followed for biocides uses with some adaptations.

- The higher tier assessment presented in the EFSA Bee guidance may not be directly suitable for the bee risk assessment for biocides since standard higher tier field studies designed for PPPs may not represent real use conditions for biocides.

3. Overview of the risk assessment

3.1. Implementation of the SPG in the risk assessment including tiered approach

3.1.1. Exposure Assessment Goal and Effect Assessment Goal

According to EFSA Bee guidance, *the implementation of the agreed SPGs in the risk assessment requires the combined evaluation of the exposure generated by the use of a PPP in the field (which can be predicted, simulated, or measured) and of the ecotoxicological effects (which are assessed as part of the hazard characterisation based on an imposed exposure in the laboratory or higher tier effect experiments).*

The same principles can be applied to the risk assessment of bees from the use of biocides. To define what exposure and which ecotoxicological effects should be used to implement the SPGs, the concepts of Exposure Assessment Goal (ExAG) and Effect Assessment Goal (EfAG) have been transposed from the EFSA guidance.

The ExAGs relate to the definition of the environmental exposure, type and duration, while the EfAGs relate to the definition of relevant model species and type of toxicity endpoints.

The definition of the ExAG allows to answer questions such as

- *where, in which matrix and for what time frame the exposure should be estimated, or*
- *what level of conservativeness the exposure estimate should aim for, i.e., what percentage of the exposure situations in the field should be covered in the risk assessment?*

The definition of the EfAG allows to answer questions such as

- *what should be the measured endpoints for the relevant species;*
- *what extrapolation approaches should be used to cover other species, endpoints and untested exposure regimes, or*
- *which percentile of a probabilistic effect assessment should be selected?*

According to EFSA Bee guidance, bees will experience various levels of exposure due to temporal differences (e.g., the same hive/nest may experience different exposure level in spring or during summer) or due to spatial differences (e.g., different hives/nests placed at different locations in the area of use of the active substances). Therefore, it is necessary to define the Exposure Assessment Goal, which can be determined by selecting a percentile that will result in realistic worst-case exposure estimation from the distribution of the various levels of the exposures. Since a 90th percentile is commonly used in ecotoxicology risk assessment e.g., for the EU FOCUS surface water, EFSA Bee guidance uses the 90th percentile and this has been taken over also in the ECHA Bee guidance document.

It is noted in the EFSA Bee guidance *that exposure and the effect (or hazard) assessments should address coherently the agreed SPGs in all the tiers and thus should be completely consistent with each other. The Ecotoxicologically Relevant Exposure Quantity (EREQ) is the conceptual interface between the effect and exposure tiers. It is based on ecotoxicological*

considerations and defines the type of exposure quantity that in a mechanistic sense best explains observed effects in an ecotoxicological experiment. In general, the EREQ is defined as the residue intake per bee per time period, as given in an ecotoxicological experiment by the dose received per bee by dietary or contact exposure. On the exposure side, the EREQ can be quantified in form of the Predicted Exposure Quantity (PEQ), for example given for dietary exposure by pesticide intake per bee per time period determined from consumption and concentration in nectar and pollen. Likewise, for contact exposure, the PEQ can be determined from respective exposure calculations. In the risk assessment, PEQ values are used then as input for effect calculations. The definitions of the key terms used in the risk assessment of bees are summarised in Table 4.

Table 4: Definition of key terms in the risk assessment of bees as defined in the EFSA Bee guidance.

Terminology	***Explanation***
EREQ ***Ecotoxicologically Relevant Exposure Quantity***	*Not a value, but a type of quantity, that gives the best mechanistic link between exposure and effects in an ecotoxicological experiment, and that is calculated/estimated both in the field[5] (PEQ) and the ecotoxicological tests (dose)*
PEQ ***Predicted Exposure Quantity***	*A value, i.e., the quantification of an EREQ for a specific compound in the field/ area of use.*
Dose	*A value: administered exposure in laboratory ecotoxicological tests*
EED, Estimated Exposure Dose	*A value: estimated in effect field studies*

3.1.2. Tiered approach for biocides

The tiered approach for biocides is largely following the principles of tiered approach established by the EFSA Bee guidance: *with ExAGs and EfAGs, both exposure estimation and effect assessment can be performed following a tiered approach. The concept of tiered approaches is to start with a simple assessment such as a screening assessment and add reality and complexity by moving to Tier 1, Tier 2, or higher tier, if necessary to refine the risk i.e., when the risk is not excluded at screening or the lower tier*. A fundamental aspect of the tiered approach in the EFSA Bee guidance is that every Tier of the exposure assessment should address the same ExAG and every Tier of the effect assessment should address the defined EfAG. Therefore, the same EREQ should be addressed in all tiers.

Both the ecotoxicological endpoints and the exposure in the area of use or the area contaminated should be expressed as the same type of exposure quantity (e.g., µg/bee per day) in order to enable a consistent linking between each effect and exposure assessment tier. Since both the exposure and effect assessments are operationalised in the tiered approach, it is appropriate to define an exposure-tier and an effect-tier:

- Exposure-Tier: In the exposure tiers, residue intake or residue deposition need to be quantified by calculating the PEQ to address the dietary and the contact route of exposure

[5] For biocides, the exposure is calculated in the area of use

of the bees following the use of a biocidal product.

- Effect-Tier: In the effect tiers the imposed exposure is called 'Dose' in the laboratory tests or 'Estimated Exposure Dose' in the higher tier tests.

For both exposure and effect assessment, the bee routes of exposure should be addressed by considering the different timescale of effects (acute and chronic) and the different life stages (adults and larvae). To this purpose in this guidance document four risk cases have been defined:

- *Acute-contact risk*
- *Acute-dietary risk*
- *Chronic-dietary risk*
- *Larvae-dietary risk*

The exposure estimation in the different Tiers will provide PEQ for each of the above risk cases for considered exposure release routes/exposure scenarios *and it is indicated as PEQ_j, where the suffix j indicates the four risk cases*. In parallel, the effect assessment in the lower Tier will provide *dose-responses for the different timescales of the effect (acute and chronic) and different life stages (adult and larvae) and therefore address the four risk cases.*

In the lower tiers of the exposure assessment, the exposure estimation is based on default parameters, while in higher tiers the exposure of the colony (or population) may be based on measured parameters (e.g., concentrations measured at the plant or brought into the hive/nest by bees). Similarly, in the lower tiers, the effect or hazard assessment is based upon ecotoxicological experiments with individual bees in laboratory studies, while the highest tier is formed by different type of studies e.g., semi-field, colony feeder and/or field tests.

For biocides, the first tier is generally conservative and based on laboratory toxicity data and exposure estimations based on default values. If the risk is found not acceptable at first tier, the applicant is offered the opportunity to submit additional information for conducting a refined risk assessment. This guidance follows in general the concepts from EFSA's tiered approach but includes simplifications and assumptions assuming different level of information and exposure patterns relevant for biocides, see Figure 2.

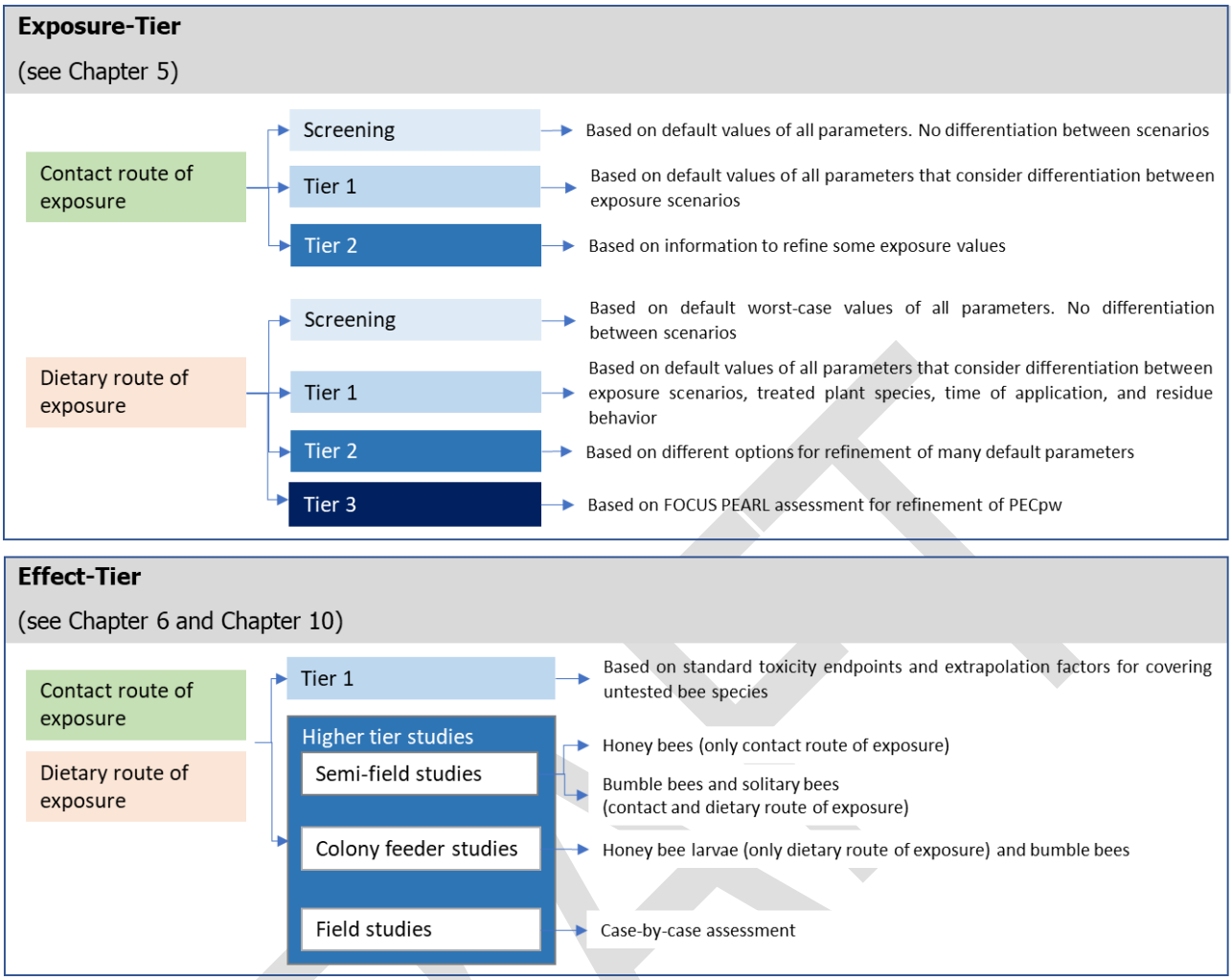


Figure 2: ***Tiered approach and explanations what each exposure or effect tier implies for the risk assessment of an active substance. According to the principle of the tiered approach, each exposure tier can be linked to each effect tier*****. FOCUS = FOrum for the Co-ordination of pesticide fate, PEARL = Pesticide Emission Assessment at Regional and Local scales, PECpw = Predicted Environmental Concentration in pore water.**

3.2. Risk assessment scheme

In this section, the bee risk assessment scheme for biocides is described, which follows the risk assessment scheme for PPPs presented in EFSA Bee guidance. The flowchart as presented in Figure 3 gives on overview of the risk assessment referring to honey bees (for the lower effect tiers). Nevertheless, the scheme may be applied also to bumble bees and solitary bees. However, for those two bee categories, the risk assessment cannot be concluded because the calculated risk cannot be compared to the corresponding SPG since no SPG could be defined for bumble bees and solitary bees (see above).

In case the risk remains unacceptable after exposure refinement and no appropriate risk mitigation measures can be applied, higher tier effect studies would be the last option to refine the risk (see Chapter 10). If an applicant intends to perform higher tier effect studies for biocides in order to show acceptable risk for bees, the applicant shall consult the evaluating competent authority during the preparation phase of the dossier and prior to conducting such tests. Together with the evaluating competent authority, appropriate test conditions and study design should be discussed and defined, as the standard higher tier effect studies designed for PPPs might not be applicable for biocides.

In Chapter 4, it is described for which types of biocidal applications a quantitative risk assessment is needed. Therefore, when *exposure to bees cannot be excluded, exposure estimation and effect assessment should be performed in order to identify the most relevant endpoints for risk assessment (see Chapters 5 and 6, respectively). Based on the exposure estimations and effect assessment, the worst-case PEQj and the relevant endpoints for each of the four risk cases needs to be selected.*

In line with the EFSA Bee guidance, for the lower tier risk assessment, a combined approach which will integrate the four different risk cases is presented. The approach is described in Chapter 7; it allows to calculate the predicted individual level effect (PIE_j) for each worst-case PEQ_j, on the basis of the selected dose-response curve (see Chapter 6). The individual effects are extrapolated to the colony/population level effect (PEQ_j) based on 1:1 relationship and then they are combined to predict the overall effect at colony/population level (PE_{SPG}), which is directly compared to the SPG which is the threshold of acceptable effects.

The lower tier risk assessment scheme starts with the screening step or Tier 1 exposure assessment and proceeds to the next exposure-tier when unacceptable risk is identified. When an exposure-Tier 2 assessment is needed, applicants should refine the exposure emission in Tier 1 as proposed in Chapter 5 of this guidance.

If an unacceptable risk cannot be concluded based solely on the exposure refinement (i.e., exposure-Tier 2 or 3), a higher effect-tier assessment may be required.

Alternatively, risk mitigation measures could be proposed by the applicant at exposure tier 1 or higher, but not for the screening step (see Chapter 13).

Furthermore, as part of the effect-tier assessment of biocidal products, three additional aspects should **in parallel** be addressed at lower tier:

- *Time-Reinforced Toxicity (TRT) assessment (Chapter 8):* the potential for the substance under evaluation for showing increasing toxic effects due to long-term exposure to low doses;
- *Sublethal effects (Chapter 9):* the potential concerns due to sublethal effects; and
- *Warning sentence (Chapter 13.3)*: the need to apply a warning sentence for biocidal products

TRT assessment is determined via extrapolation from the standard 10-day chronic honey bee toxicity study (OECD 245). Regarding sublethal effects, the Tier 1 allows to identify potential concerns which should be addressed with further testing after consulting the evaluating competent authority. The evaluation of TRT and sublethal effects is for the time being only performed for honey bees.

The warning sentence is based on the hazard properties of the active substance and is warranted for the biocidal product if certain criteria are met.

As part of the risk assessment scheme, also the risk from **metabolites** should be addressed. A risk assessment scheme for metabolites is presented in Chapter 11.

Also, an approach for the risk assessment of **mixtures** is provided in Chapter 12. The risk assessment of mixtures is performed in case the biocidal product contains more than one active substance.

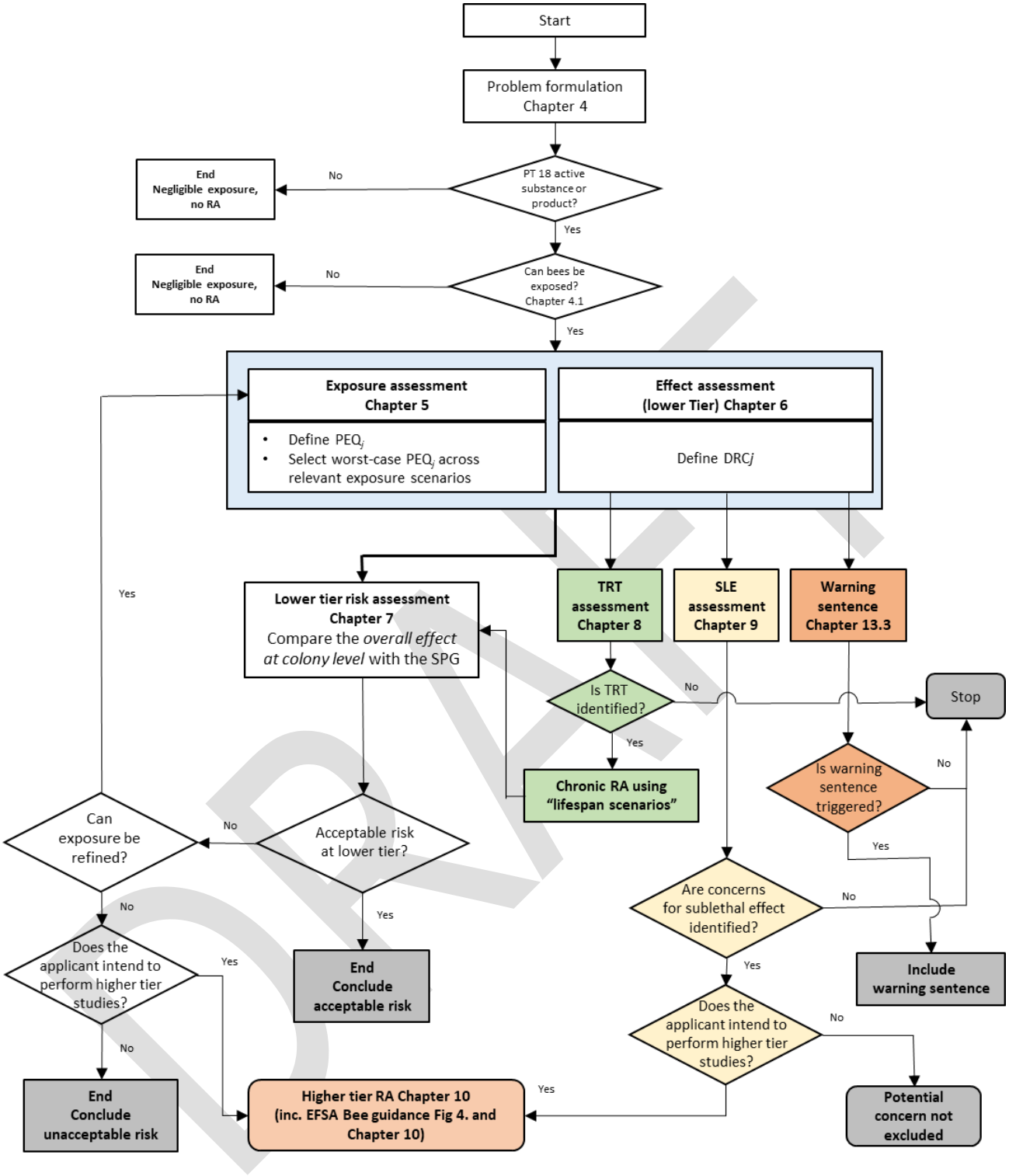


Figure 3: Overview of the biocide lower tier risk assessment scheme for honey bees (HB) for biocides. RA = risk assessment, PEQ_j= Predicted exposure quantity for the four risk cases (indicated by the suffix *j*, i.e., acute-contact, acute-dietary, chronic-dietary and larvae-dietary), DRC_j =dose-response curve for the risk case *j*, TRT= time reinforced toxicity (only relevant for HB), SPG = specific protection goal, eCA = evaluating competent authority. Note that also a RA for metabolites (Chapter 11) and for mixtures (Chapter 12) need to be conducted, where relevant. For higher tier risk assessment, see Chapter 10.

4. Problem formulation

As outlined in the EFSA guidance, *problem formulation is the first step of the risk assessment which allows applicants and risk assessors to identify the potential hazard and exposure pathways for a PPP and to formulate risk hypotheses and identify the proper risk assessment methodology. The problem formulation sets the boundaries for risk assessment such that it is 'fit for purpose'. For a risk to occur, it entails an exposure to a PPP which result in a direct harm to the bees that exceeds a specified SPG.*

When evaluating a biocide, the first step of the risk assessment is to determine through a problem formulation, if and how emissions of the biocide could reach bees; and estimate the level of the exposure (see Chapter 5). Therefore, the starting point is a careful consideration of the source of exposure which includes an analysis of the method of application or form of release, the area where the biocide is applied/released, the number of applications, the application/release rate, and any particular conditions of use.

An overview of sources of exposure from the use of biocides covered by this guidance is reported in the Table 5 below together with a consideration of the relevance of the routes of exposure (see Section 1.4) and exposure scenarios (see Section 4.1).

Table 5: Overview of the possible sources of exposure in relation to the contact and dietary routes of exposure in the exposure scenarios

	Contact			Dietary			
	Treated area	**Weeds in the treated area**	**Vegetation margin**	**Treated area**	**Weeds in the treated area**	**Vegetation margin**	**Plants in treated area during the next growing season**
Application of manure/sludge to agricultural soil or grassland	N	N	N	Y	(Y)	N	(Y)
Spraying of walls and foundation of houses	N	N	Y	N	N	Y	N
Irrigation of private gardens	Y	(Y)	N	Y	(Y)	N	(Y)
Outdoor large scale spraying	Y	Y	Y	Y	Y	Y	Y

(Y) = scenarios considered covered by another worst-case scenario since either sugar content of the vegetation covered by the scenario is higher or equal to that of the worst case scenario and/or exposure models result in the same results as for the worst case scenario.

4.1. Exposure scenarios

Bees may be exposed in the treated areas (i.e., treated area and weeds in the treated area scenarios) and/or in the surrounding areas (i.e., vegetation margin and adjacent area scenarios). Furthermore, in some situations, bees may be exposed to residues in pollen and nectar that are taken up by the plants in the next growing season. In those scenarios bees may be exposed by

contact and/or by dietary routes.
In relation to the contact exposure, it is considered that bees can be over-sprayed in the treated areas and/or could come in contact with spray drift in the surrounding areas at the time of the application.

In relation to the dietary exposure via consumption of contaminated pollen and nectar, the EFSA Bee guidance notes that *the proportional contribution of the various exposure scenarios to the daily food intake by bees is unknown*. Therefore, it is assumed *that each scenario contributes to 100% of the contaminated food consumed by bees, as worst-case*.

Further, according to the EFSA Bee guidance, *among the most relevant scenarios, only those scenarios that will strongly dominate the exposure on the basis of the exposure estimation will be used for risk assessment, since it is considered to cover all the others. This means that worst-case PEQj will be selected across scenarios for risk assessment (see Chapter 7) and the worst-case PEQj should be identified at each tier of the risk assessment.*

Below is a description of the exposure scenarios that have been adapted from the EFSA Bee guidance.

4.1.1. Treated area scenario

The exposure of bees to biocides in treated areas entails that bees visit and interact with plants; therefore, it is necessary to ascertain whether the plants are attractive to bees. As pollen and nectar are the main sources of nutrition for bees, the attractiveness is based on the presence and availability of pollen and nectar. A list of plants/crops that are attractive to bees is presented in Appendix A of the EFSA Bee guidance. Note that when a plant is attractive to bees, contact exposure and dietary exposure cannot be excluded. When the treated area does not contain attractive plants, the exposure is assumed to be zero and therefore the treated area scenario is not relevant.

For biocides, the "treated area" may consist of trees or bushes subject to biocide treatment such as large scale spraying against mosquitoes or processionary moths. The trees could be treated in a row or as solitary trees. In case of treated trees, it is the crown of the tree (i.e., the branches, leaves, and reproductive structures extending from the trunk or main stems) that determine the borders of the treated area.

4.1.2. Weeds in the treated area scenario

When the 'treated area' scenario consists of treated plants/area that is considered not attractive/not relevant for bee exposure, bees may still be exposed in the treated areas while foraging on the flowering weeds present in those areas. For biocides, "weeds" may include also bushes, flowers, grass, or berries that grow within the treated area.

4.1.3. Vegetation margin and adjacent area scenarios

Areas surrounding the treated area can be defined as 'vegetation margin' and 'adjacent area'. "Vegetation margin" may be park lawns, meadows, or countryside roads located at the edge of the treated area, forest, or tree crown. The vegetation margins are assumed to consist of mixed vegetation that is flowering at the time of application. "Adjacent area" may be meadows, countryside roads or fields. The adjacent areas are also assumed to be covered by various plants/mixed vegetation which are flowering at the time of application. The vegetation in these areas is exposed by spray drift (spray application). The exposure of bees in the adjacent area is considered to be lower than in the vegetation margin and therefore only vegetation margin scenario may be relevant (for e.g., large scale spraying).

The vegetation margin has to be considered a relevant exposure scenario for both the contact and the dietary routes of exposure, since this represents a relevant area of interest for bee

habitats. For biocides applications, it is assumed, that the vegetation margin is always downwind.

4.1.4. Plants in treated area during the next growing season

In the "Plants in treated area during the next growing season" scenario, bees are exposed to pollen and nectar contaminated with residues of the active substance that are already present in the soil following a previous treatment. Residues that persist in soil are taken up by the roots of plants and then translocated via the vascular system and the tissues of plants to nectar and pollen. This may happen for plants that are cultivated twice in a growing season or the following year. Residues may be found in the pollen and nectar of for example treated trees and bushes in the following year. It is assumed that these plants are attractive for both pollen and nectar.

5. Exposure assessment

Exposure of bees to biocides can occur, when biocidal products are intentionally applied outdoors or if matrices that were unintentionally contaminated with biocidal active substances (e.g., manure or sewage sludge) are released to the outdoor environment. Biocides might then reach the flowers of plants directly or can be taken up by the plants from soil and can accumulate in their pollen and nectar (see Figure 4). Hence, during consumption of pollen and nectar, bees take up the active substance orally. Furthermore, contact exposure of bees to biocides is possible when bees come in direct contact to the biocide (e.g., droplets of spray drift) or are exposed to contaminated matrices (e.g., spray deposition of biocides on plants).

Further exposure routes which are not considered in this document, but might become relevant once more knowledge has been acquired, are:

- the consumption of contaminated water (e.g., puddles formed during spraying, contaminated surface water, or guttation water),
- the consumption of other contaminated plant matrices (e.g., honey dew, extrafloral nectaries, resin, wax etc.)
- contact exposure of bee species which are breeding in contaminated soil or wood, or which use other contaminated materials (e.g., leaves, mud) to build their brood cells

Therefore, in alignment with the EFSA Bee guidance only the main exposure pathways via the consumption of pollen and nectar (oral exposure) or via direct contact to the biocide or contact to contaminated plant surfaces are considered, if relevant, for the identified sources of exposure to biocides.

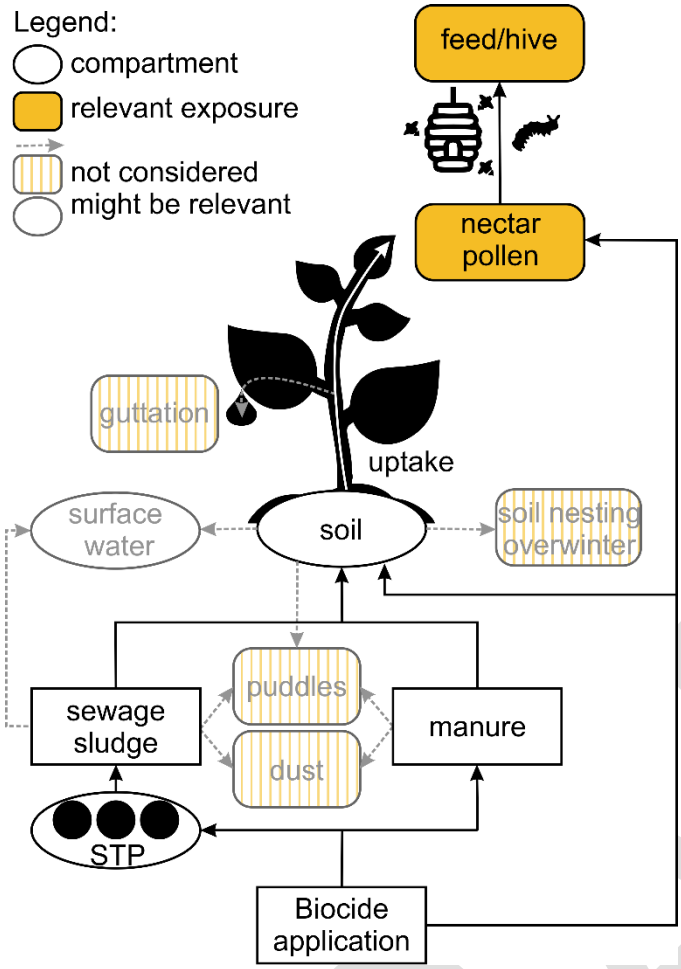


Figure 4: The main exposure pathways of bees to biocides via the consumption of pollen and nectar (oral exposure) are shown. Shaded are further exposure pathways that could become relevant once more knowledge has been acquired.

This chapter describes 1) the mathematical models to be used for the estimation of exposure of bees for the different exposure routes to be covered by the risk assessment in general; and 2) the relevant exposure pathways and applicability of the mathematical models for the exposure estimation by each relevant biocides source of exposure.

For the biocides sources of exposure presented in sections 5.2-5.7, the input and output data and calculations are specified in the tables 12 to 39. The input and output data are divided into four groups:

S Set	Parameter must be present in the input data set for the calculation to be executed (no method has been implemented in the system to estimate this parameter; no default value is set, data either needs to be supplied by the applicant or should be available in the literature).
D Default	Parameter has a standard value (most defaults can be changed by the user).
O Output	Parameter is the output from another calculation (most output parameters can be overwritten by the user with alternative data).
P Pick list	Parameter value can be chosen from a "pick list" of values.

Pick list values and default parameters are to be adapted, when specific data is available, instead of a mandatory use of these values as defaults.

It should be noted the input data to be used for the purposes of the assessment of risk to bees should be in line with the input data used in the exposure assessment (soil/surface water) for given case under evaluation.

5.1. The exposure assessment models

The exposure assessment models that are described in this chapter showcase the mathematical expressions of the exposure assessment. They are to be used to estimate the exposure quantity of an individual bee through the two main routes of exposure: contact and dietary exposure to biocides. As stated in the EFSA Bee guidance, these predictions are to be used in the lower tier risk assessments.

The two main routes of exposure (dietary and contact) lead to three relevant exposure assessment models for biocides: dietary above-soil model (during flowering), dietary through-soil model and contact model. The scope, applicability, and parameters of the dietary and contact models are described in more detail in Sections 5.1.1 and 5.1.2, respectively.

5.1.1. Dietary models

The dietary models are to be used when adult bees or bee larvae come into contact with the biocidal product by directly consuming the contaminated pollen or nectar. Three dietary models have been described in the EFSA Bee guidance to assess the exposure of bees, allowing the estimation of the quantity of the biocide intake by an individual:

- The dietary model for during flowering contamination, which *predicts the residue intake for during flowering applications i.e., when direct contamination of pollen and nectar is involved.*
- The dietary model for pre-flowering contamination, which is intended *for situations when the contamination of pollen and nectar dominantly originates from contamination of the above-soil parts of the treated/contaminated plants before their flowering stage*.
- The dietary model for through soil contamination, which *predicts the residue intake when the contamination of pollen and nectar originates from the residue uptake process from soil.*

The dietary models are briefly introduced below. For more detailed information on the dietary route of exposure models, see Section 5.1.2 of the EFSA Bee guidance.

The dietary model for the during flowering contamination is to be used (for all risk cases) when the open flowers of the treated/contaminated plants might directly be contaminated by the biocide. The model has been set up in the following way:

$$PEQ_{di} = \frac{AR}{1000} \times EF_{di} \times \left(SV_{po,du} + SV_{ne,du}\right) \qquad \textbf{Equation 1}$$

The shortcut value (SV) parameters are derived with the following expressions:

$$SV_{po,du} = \frac{1}{1000} \times LF_{po} \times PCUD_{po,du} \times CMP_{po}$$ **Equation 2**

$$SV_{ne,du} = \frac{1}{1000} \times LF_{ne} \times PCUD_{ne,du} \times \frac{CMP_{su}}{SN}$$ **Equation 3**

The dietary model for through soil contamination is to be used (for all risk cases) when the plants can only be contaminated via the soil. The model has been set up in the following way:

$$PEQ_{di} = SV_{po,\text{soil}} + SV_{ne,soil}$$ **Equation 4**

The SV parameters are derived using the following expressions:

$$SV_{po,\text{soil}} = \frac{1}{1000} \times LF_{po} \times PEC_{pw} \times CMP_{po}$$ **Equation 5**

$$SV_{ne,soil} = \frac{1}{1000} \times LF_{ne} \times PEC_{pw} \times \frac{CMP_{su}}{SN}$$ **Equation 6**

According to the EFSA Bee guidance, the *dietary model for pre-flowering contamination is to be used when the treated/contaminated plant, even though it is not yet in its flowering stage, might still be contaminated by the applied substance*. Since for biocides applications it is unlikely to know the timing of the application in relation to flowering of the treated/contaminated plants and/or the treated/contaminated plants are mixed and therefore there may always be flowering plants in the affected areas, the dietary model for pre-flowering contamination is not considered relevant for biocides and therefore also not further described in the ECHA Bee guidance. The dietary model for the during flowering contamination as the only model in this ECHA Bee guidance to address the dietary exposure of bees due to above soil contamination is referred to as a dietary above-soil model.

The parameters of the above models are described below.

Parameter description:

Input parameters

AR — Application rate (g/ha).
This parameter is established and named in line with the EFSA Bee guidance. For biocides, AR refers to the mass of a biocidal active substance applied or released to an area with flowering plants assuming 100% deposition.
Depending on the source of exposure, this definition can deviate from the definition of AR in a biocidal product assessment, where AR e.g., refers to a mass of a biocide per treated surface. In these cases, the emission of the biocide from the treated surface to the surrounding area with flowering plants in mass per area assuming 100% deposition is considered as AR in this guidance. In other cases, the biocide is directly applied to flowering plants and the AR can be used directly in the calculation.

CMP — Consumption of sugar (CMP_{su}) or pollen (CMP_{po}) (mg/bee/day or mg/larva/developmental period).
Consumption rates refer to the active period of bees during the flowering period. CMP values are specific for bee categories. Values are tabulated in the EFSA Bee guidance, Supplementary document.

Table 6: Food consumption rates of bees during the active period

Category for the risk assessment	Representative species and bee role category	Daily sugar consumption (mg/bee/day) or over developmental period (mg/larva/developmental period)	Daily pollen consumption (mg/bee/day) or over the development period (mg/larva/developmental period)
Honey bee	*Apis mellifera* forager	acute: 78.9* chronic: 74.7*	0
	A. mellifera nurse	34	11.6
	A. mellifera larva	81.5	1.99*
Bumble bee	generic model bumble bee species	acute: 79.8* chronic: 75.6*	11.7
	Bombus terrestris larva	194.6	60.23
Solitary bee	generic model solitary bee species	acute: 4.27* chronic: 4.05*	0.6
	Osmia bicornis larva	91	91.3*
	O. cornuta larva	165	91.3*

* Represents the 90th percentile value of the range and was used for shortcut value calculations.

EF_{di} Exposure factor for dietary exposure (-).
EF_{di} represents the proportion of the applied chemical that deposits on the plants due to spray drift or dust formation including a safety factor. It is dependent on the spray drift/dust drift (if relevant) and the growth stage of the crop/plants and the resulting interception. Default values for EF_{di} are tabulated in EFSA Bee guidance Appendix B.

LF Landscape dilution factor for pollen (LF_{po}) and nectar (LF_{ne}) (-).
This factor accounts for potential dilution in the residue levels entering the hive. An LF of 1 would mean that 100% of the food entering the hive/nest originates from contaminated pollen/nectar.
It is considered that the landscape factor for the acute exposure assessments for pollen and nectar should be 1 (100% of the collected pollen origin from the contaminated crop). A LF <1 is recommended for the chronic dietary exposure estimation in pollen for honey bee adults and larvae for the scenarios 'Treated area' and 'Plants in treated area during the next growing season' when a single plant species is assumed to be growing in the given area. The LF is not a single value, but a range of values that feeds into the Monte Carlo exposure estimation.

PCUD Predicted Concentration per Unit Dose in pollen ($PUCD_{po,du}$) and nectar ($PUCD_{ne,du}$) from during flowering application (mg/kg).

PEC_{pw} Predicted Environmental Concentration in pore water (mg/kg = mg/L). PEC_{pw} parameter for the Tier 1 exposure estimation is a single default value, which is 1 mg/L.

SN Sugar content in nectar expressed as mass/mass (e.g., kg/kg). The sugar content of the nectar is crop/plant-dependent, lower sugar contents result in higher nectar consumption by bees. EFSA defines SN values for several crops. For biocides, the crop/plant species is often unknown, therefore, the default SN of 10% for solitary bees and 15% for honey bees and bumble bees applies. In

habitats with mixed vegetation, a sugar content of 30% is to be considered for the risk assessment. In cases where the crop/plant species is known, the EFSA Bee guidance Table 22 can be used. Otherwise, the following values from Table 7 should be used.

Table 7: Sugar content in nectar

Vegetation type	Sugar content in nectar (%)		
	Honey bee	Bumble bee	Solitary bee
Unknown crop/ grassland or Treated specific species of vegetation (not listed in EFSA Bee guidance Table 17)	15	15	10
Mixed vegetation	30	30	30

SV Shortcut value (µg/bee or µg/bee/day or µg/larva/developmental period). SVs represent the 90th percentile of the distribution of the residue intakes by bees, based on an application rate of 1 kg a.s./ha. SVs have been calculated with a Monte Carlo method and are tabulated by EFSA (see EFSA Bee guidance Appendix B). The values are classified according to the type of food (nectar or pollen), role of the bee (forager acute/chronic, nurse acute/chronic or larvae), period of flowering (before or during application), and exposure pathway (spraying (downward or sideward/upward, via soil). Although SVs are very PPP specific, ECHA EG decided that certain SVs can be applied for biocides as a worst-case approach, considering that the AR for biocides is usually << 1 kg a.s./ha. Relevant SVs for biocides are shown in Appendix B.

Table 8: Overview shortcut values

Parameter	Definition
$SV_{po,du}$	Shortcut value for pollen for during flowering situations
$SV_{ne,du}$	Shortcut value for nectar for during flowering situations
$SV_{po,soil}$	Shortcut value for pollen for situations for contamination from soil
$SV_{ne,soil}$	Shortcut value for nectar for situations for contamination from soil

Output parameters

PEQ_{di} Predicted Exposure Quantity due to dietary exposure (µg/bee or µg/bee/day or µg/larva/developmental period).
PEQ_{di} refers to the intake of biocide mass per bee.
For the chronic adult assessments, this quantity has to be expressed per day, but for the larvae it has to be expressed as the sum of the intake over the entire developmental period.

For detailed information, refer to EFSA Bee guidance Chapter 5.

5.1.2. Contact model

The contact model is to be used when there is *physical contact between the surface of the bee and the biocide. This route of exposure may take place during or shortly after the spray application of the biocidal product. Thus, it is mainly relevant for foraging honey bees, foraging worker bumble bees and adult solitary bees*. For more information on the contact route of

exposure models, see Section 5.1.1 of the EFSA Bee guidance.

The model to be used in the lower tier exposure assessment, as stated in the EFSA Bee guidance is the following:

$$PEQ_{co} = AR \times EF_{co} \times BSF$$ **Equation 7**

The parameters of this model are described below.

Parameter description:

Input parameters

AR Application rate (g/ha) (see Chapter 5.1.1).

BSF Body surface factor (dm²/bee).
BSF is applied to take into consideration the size differences between bee species when performing the exposure assessment.

Table 9: Body surface factor

Category for the risk assessment	Representative species	BSF (dm²/bee)
Honey bee	*Apis mellifera*	0.0114
Bumble bee	5th percentile (by body surface) bumble bee species	0.0146
Solitary bee	5th percentile (by body surface) solitary bee species	0.00184

EF_{co} Exposure factor for contact exposure (-).
EF_{co} represents the proportion of the applied chemical that deposits on plants due to spray drift or dust. It is dependent on the spray drift/dust drift (if relevant) and the growth stage of the crop/plants and resulting interception. Default values for EF_{co} are tabulated in the EFSA Bee guidance Appendix B.

Output parameters

PEQ_{co} Predicted Exposure Quantity for contact exposure (µg/bee).

For detailed information, refer to EFSA Bee guidance Chapter 5.

5.1.3. Screening step

The exposure assessment for the dietary route of exposure is a complex process, involving several steps and numerous parameters. Therefore, EFSA has formed a simplified method for the derivation of PEQ_{di} values. This screening PEQ_{di} can be used in the combined risk assessment (see Chapter 7). Applying this screening is an option, but not mandatory and can only be used as far as the cumulative application rate (AR x n) is not higher than 4.5 kg/ha. The method for PEQ_{di} derivations for the screening step is a simplified version of the models described in Sections 5.1.1 and 5.1.2, resulting in conservative exposure estimations compared to Tier 1. In the simplified model for the during flowering contamination model, application rate (AR; g/ha), and

the number of 38ffect38tionns (n) has to be combined with a constant B in the following way:

$$PEQ_{di} = \frac{AR}{1000} \times n \times B \qquad \textbf{Equation 8}$$

where B is a constant that depends on the risk case and the application method as presented in Table 10. Where spraying technology for biocides applications is not matching with agricultural spraying techniques, or where details of the spraying technique would not be available during the assessment, constant B for sideward/upward spray application is to be used as a worst case.

Table 10: ***The values of constant B (µg/bee or µg/bee/day or µg/larva/developmental period) for each risk case by application methods***

Category for the risk assessment	***Risk case***	***Constant B to be used for downward (DW) spray***	***Constant B to be used for sideward/upward (SUW) spray application***
Honey bee	*acute adult*	*6.4*	*9.0*
	chronic adult	*6.2*	*9.0*
	larva	*7.2*	*9.2*
Bumble bee	acute adult	*10*	*13.7*
	chronic adult	*9.6*	*13.3*
	Bombus terrestris larva	*33.7*	*48.5*
Solitary bee	acute adult	*0.70*	*0.94*
	chronic adult	*0.67*	*0.90*
	Osmia bicornis larva	*38.2*	*57.5*
	Osmia cornuta larva	*47.2*	*68.8*

This method results in more conservative PEQ_{di} values for all situations which would be calculated by the model for the above soil contamination.

As regards the through soil contamination, single default PEQ_{di} values are available, which are independent of the application rate as presented in Table 11.

Table 11: The PEQ_{di} values (µg/bee or µg/bee/day or µg/larva/developmental period) relevant for situations or scenarios where the through soil contamination model is to be applied

Category for the risk assessment	***Risk cases***	***PEQdi***
Honey bee	*acute adult*	*0.530*
	chronic adult	*0.500*
	larva	*0.542*
Bumble bee	*acute adult*	*0.541*
	chronic adult	*0.511*
	Bombus terrestris larva	*1.357*
Solitary bee	*acute adult*	*0.044*
	chronic adult	*0.041*
	Osmia bicornis larva	*0.993*
	Osmia cornuta larva	*1.783*

The next step is to compare the PEQ_{di} values calculated by applying Equation 8 with the PEQ_{di}

values reported in Table 11. For each risk case, the highest of the two PEQ_{di} values has to be considered in the risk assessment for the screening step.

As regards to the screening step for the contact exposure, the PEQ_{co} is calculated using the following simplified model:

$$PEQ_{co} = AR \times BSF$$ **Equation 9**

The units of the parameters are the same as described in section 5.1.2.

5.2. Source of exposure – manure/sewage sludge application

5.2.1. Description of source of exposure

This section concerns the assessment of exposure and risk to bees due to application of manure (including slurry) or sewage sludge on agricultural soil and grasslands. Following the consideration that the exposure of bees would not be negligible for this release route, it is required to assess risk for bees. Exposure of bees to biocidal active substances due to the application of manure or sewage sludge is considered to be relevant for the following emission scenario for biocides:

- PT18: Insecticides used in Stables and Manure Storage Systems – Emission to Manure
- PT18: Insecticides used in Stables and Manure Storage Systems – Emission to sewage sludge: Regarding application of sewage sludge on soil, the risk assessment for bees needs to be performed for releases of biocides to municipal STP after treatment of animal housings for animal subcategories i8, i11-12 and i16-18 with biocides (ESD PT18 for Insecticides for stables and manure storage systems 2006).

Following the application of the biocidal product in animal housings or manure storage systems, the biocidal active substance can, depending on the release pathway, reach manure or sewage sludge (see ESD PT18 (2006)), which might be emitted to the environment either via the spreading of manure or through the application of sewage sludge on agricultural soil or grassland. Active substances and their metabolites can then be taken up by plants growing on the agricultural soil/grassland and translocated via the vascular system and the tissues of plants to pollen and nectar. From there they can be taken up and consumed by bees.

Bees may be mainly exposed to biocides in manure/sludge via the consumption of contaminated pollen and nectar (oral exposure) after spreading of manure or sewage sludge on agricultural land or grassland.

The main exposure scenario that needs to be addressed for this source of exposure is the Treated area scenario covering the Weeds in the treated area and Plants in treated area during the next growing season as a worst-case. The exposure of bees relies on the dietary model for through soil contamination. The Vegetation margin scenario is not relevant for this source of application as there is no spray drift.

5.2.2. Dietary exposure model

5.2.2.1. Tier 1

Tier 1 is based on default shortcut values for "through soil contamination" from the EFSA Bee guidance.

Specific input parameters for Tier 1

$SV_{po,soil}$ The shortcut value is based on a PEC_{pw} of 1 mg/kg, which can be considered as an extreme

worst-case for biocides. SVs for pollen for through soil contamination apply, see Appendix B.

$SV_{ne,soil}$ The shortcut value is based on a PEC_{pw} of 1 mg/kg, which can be considered as an extreme worst-case for biocides. SVs for nectar for through soil contamination apply, see Appendix B.

0

Calculations for Tier 1

Table 12: Tier 1 calculations for dietary model for through soil contamination

Parameters	Nomenclature	Value	Unit	Origin	Source
Input					
Shortcut value for pollen for through soil contamination	$SV_{po,soil}$		µg/bee or µg/bee/day or µg/larva/developmental period	P	Appendix B
Shortcut value for nectar for through soil contamination	$SV_{ne,soil}$		µg/bee or µg/bee/day or µg/larva/developmental period	P	Appendix B
Output					
Predicted Exposure Quantity due to dietary exposure	PEQ_{di}		µg /bee or µg /bee/day or µg /larva/developmental period	O	
Calculation					
$PEQ_{di} = SV_{po,soil} + SV_{ne,soil}$ **Equation 4**					

5.2.2.2. Tier 2

Basically, the same calculation as in Tier 1 is conducted but the shortcut values are re-calculated based on a product specific PEC_{pw}, which is a standard output value of the environmental exposure assessment for manure or sewage sludge application and can be derived according to Guidance on BPR, Vol. IV, Part B+C (2017, p. 93, equation 70).

Specific input parameters for Tier 2

$PEC_{pw,2}$ Predicted environmental concentration in porewater after application of manure or sludge [$mg \times L^{-1}$]. Prior to calculating a PEC_{pw}, a PEC_{soil} needs to be derived. PEC_{soil} is calculated as the time-weighted average (TWA) concentration in soil over 180 days after the last application on land after 10 consecutive years of manure or sludge application (TAB ENV 237 (2022)).

Calculations for Tier 2

Table 13: Tier 2 calculations for dietary model for through soil contamination

Parameters	Nomenclature	Value	Unit	Origin	Source
Input					
Predicted Environmental Concentration in pore water – Tier 2	$PEC_{pw,2}$		mg/L	S	Biocides exposure assessment
Shortcut value for pollen for through soil contamination	$SV_{po,soil}$		µg/bee or µg/bee/day or µg/larva/developmental period	P	Appendix B
Shortcut value for nectar for through soil contamination	$SV_{ne,soil}$		µg/bee or µg/bee/day or	P	Appendix B

			µg/larva/developmental period		
Output					
Predicted Exposure Quantity due to dietary exposure – Tier 2	$PEQ_{di,2}$		µg/bee or µg/bee/day or µg/larva/developmental period	O	
Calculation					
$PEQ_{di,2} = (SV_{po,soil} + SV_{ne,soil}) \times PEC_{pw,2}$ **Equation 10**					

5.2.2.3. Tier 3

In Tier 3, the same calculation as in Tier 1 is conducted but the shortcut values are re-calculated based on a product-specific PEC_{pw}. The PEC_{pw} in Tier 2, which is calculated from PEC_{soil} in the biocides exposure assessment and is considered as a conservative value, can be refined by the modelling tool FOCUS PEARL. For all other input parameters, a refinement is not considered feasible as the area of application of manure or sludge is unknown and no crop specific parameters can be derived.

Specific input parameters for Tier 3

$PEC_{pw,3}$ PEC_{pw} is refined by the use of FOCUS PEARL v. 5.5.5 software. The agreed general input parameters and settings for FOCUS PEARL v. 4.4.4 according to ENV 23, 165 and 166 (TAB ENV (2022)) for modelling of groundwater concentrations are also valid for modelling of PEC_{pw}. Similar to the EFSA Bee guidance, the refined PEC_{pw} should be derived over 20 cm soil depth, and 150 days after the last application on agricultural land and 120 days after the last application on grassland. A detailed description of settings of the FOCUS PEARL v. 5.5.5 for modelling of porewater concentrations can be found in Appendix C.

Calculations for Tier 3

Table 14: Tier 3 calculations for dietary model for through soil contamination

Parameters	Nomenclature	Value	Unit	Origin	Source
Input					
Predicted Environmental Concentration in pore water – Tier 3	$PEC_{pw,3}$		mg/L	S	Biocides exposure assessment
Shortcut value for pollen for through soil contamination	$SV_{po,soil}$		µg/bee or µg/bee/day or µg/larva/developmental period	P	Appendix B
Shortcut value for nectar for through soil contamination	$SV_{ne,soil}$		µg/bee or µg/bee/day or µg/larva/developmental period	P	Appendix B
Output					
Predicted Exposure Quantity due to dietary exposure – Tier 3	$PEQ_{di,3}$		µg/bee or µg/bee/day or µg/larva/developmental period	O	

End Calculation	
$PEQ_{di,3} = \left(SV_{po,soil} + SV_{ne,soil}\right) \times PEC_{pw,3}$	Equation 11

5.3. Source of exposure – spraying on walls and foundation of houses

5.3.1. Description of source of exposure

A quantitative risk assessment for bees needs to be performed for the products applied around buildings, i.e., for spray applications onto walls for flying insects and/or foundation for crawling insects (corresponding to outdoor emission model "spray application: treatment around the building" on p. 136 of the ESD PT 18 for household and professional uses (2008)). For wall treatment, it is assumed that the whole surface of the walls of a house is sprayed, whereas for foundation treatment only the bottom wall area (0.5 m of height) is treated. Spray treatment of walls and/or foundation with biocidal products leads to the contamination of a soil strip that surrounds the treated house. The contamination occurs through spray deposition or through run-off and/or wash-off by rainfall. For the risk assessment for bees, the contaminated soil surrounding the house is assumed to be part of the garden that is covered with flowers or bushes (mixed vegetation) that are attractive to bees. Only private houses are considered relevant as commercial buildings are not assumed to be surrounded by gardens.

For both the treatment of walls and foundation, dietary (above and through soil contamination for the same contaminated soil area) and contact exposure of bees are relevant. Due to deposition from the spray application, nectar and pollen of flowering plants around the house are directly contaminated by biocides (above soil contamination). Due to run-off and wash-off by rainfall, biocides reach the soil and then they are taken up by the plants via roots (through soil contamination). Bees can also get in direct contact with the biocides due to the spray drift or contact with contaminated plant matrices shortly after the biocidal treatment (contact exposure).

The Vegetation margin scenario is considered the only relevant exposure scenario for the spray application on walls and/or foundation because bees are exposed in the area adjacent to the wall and/or foundation treated with biocides. The plants growing on the band of contaminated soil surrounding the treated house are considered mixed vegetation which is in flower at the time of application (attractive to bees). In contrast to the PPP assessment, where only above soil contamination is considered for the Field margin scenario, for this biocides source of exposure both above and through soil contamination need to be considered (see above). Since the wall or the foundation is the object of the biocidal treatment but as such Is not relevant for exposure of bees, the exposure scenarios Treated area, Weeds in the treated area and Plants in the treated area during the next growing season are not relevant for the risk assessment.

5.3.2. Dietary exposure model

5.3.2.1. Screening step

The screening step as described in Chapter 5.1.3 could be applied. If unacceptable risk is identified, the risk assessment needs to move to Tier 1. Table 15 presents the input and output parameters for the dietary model for above soil contamination.

Specific input parameters for Screening step

Q_{prod}	Quantity of product applied to treated surface
Fai	Fraction of active substance in the product
$AREA_{treated}$	Treated area for wall or foundation spraying. The default values for treated wall and

foundation areas of a private house are 125 m^2 and 25 m^2, respectively (ESD PT 18(2008)).

$AREA_{soil}$ Area of soil around the house that is contaminated during and after spraying. The width of this soil band is 0.5 m according to ESD PT 18 (2008). The contaminated soil area next to the wall and foundation is therefore considered to be 26 m^2 for private houses (see Figure 4.3-4 in ESD PT18 (2008)).

N Number of applications per year, the value is set to 1 for this source of exposure.

B Constant B. Values to be used for sideward/upward (wall) or downward (foundation) spray application are applicable for this source of exposure (see Table 10 in Chapter 5.1.3)

Calculations for Screening step

Table 15: Screening step calculations for dietary model for above soil contamination

Parameters	Nomenclature	Value	Unit	Origin	Source
Input					
Quantity of product applied (e.g., to wall, foundation)	Q_{prod}		g/m^2	S	
Fraction of a.s. in the product	F_{ai}			S	
Treated surface (e.g., wall, foundation)	$AREA_{treated}$	125	m^2	D/S	ESD PT 18 (2008)
Area of soil that is contaminated	$AREA_{soil}$	26	m^2	D/S	ESD PT 18 (2008)
Number of applications	n			S	
Constant B for SUW spray (wall) or DW spray (foundation)	B		µg/bee or µg/bee/day or µg/larva/develop mental period	P	Table 10
Output					
Application rate	AR		g/ha	O	
Predicted Exposure Quantity due to dietary exposure	PEQ_{di}		µg/bee or µg/bee/day or µg/larva/develop mental period	O	
Calculation					
$AR = Q_{prod} \times F_{ai} \times \frac{AREA_{treated}}{AREA_{soil}} \times 10000$					**Equation 12**
$PEQ_{di} = \frac{AR}{1000} \times n \times B$					**Equation 8**

As regards the dietary model for through soil contamination, single default PEQ_{di} values are available, which are independent of application rate, as presented Table 11. The next step is to compare the PEQ_{di} values calculated by applying Equation 8 with the PEQ_{di} values reported in Table 11. For each risk case, the highest of the two PEQ_{di} values has to be considered in the risk assessment for the screening step.

5.3.2.2. Tier 1

Table 16 presents the input and the output parameter for the dietary model for above soil

contamination.

Specific input parameters for Tier 1 – above soil contamination

$SV_{po,du}$ Shortcut values for pollen, sideward/upward (SUW, wall) or downward (DW, foundation) spraying are applicable taking into account that mixed vegetation is growing on the band of soil around the house, see Appendix B.

$SV_{ne,du}$ Shortcut values for nectar, sideward/upward (wall) or downward (foundation) spraying are applicable taking into account that mixed vegetation is growing on the band of soil around the house, see Appendix B.

EF_{di} Exposure factor for dietary exposure, value is set to 0.1 which equals the deposition fraction for wall treatment according to ESD PT 18 (2008).

Calculations for Tier 1 – above soil contamination

Table 16: Tier 1 calculations for dietary model for above soil contamination

Parameters	Nomenclature	Value	Unit	Origin	Source
Input					
Quantity of product applied (e.g., to wall, foundation)	Q_{prod}		g/m²	S	
Fraction of a.s. in the product	F_{ai}			S	
Treated surface (e.g., wall, foundation)	$AREA_{treated}$	125	m²	D/S	ESD PT 18 (2008)
Area of soil that is contaminated	$AREA_{soil}$	26	m²	D/S	ESD PT 18 (2008)
Shortcut value for pollen for above soil contamination – for SUW or DW spraying	$SV_{po,du}$		µg/bee or µg/bee/day or µg/larva/developmental period	P	Appendix B
Shortcut value for nectar for above soil contamination – for SUW or DW spraying	$SV_{ne,du}$		µg/bee or µg/bee/day or µg/larva/developmental period	P	Appendix B
Exposure factor for dietary exposure	EF_{di}	0.1		D	ESD PT 18 (2008)
Output					
Application rate	AR		g/ha	O	
Predicted Exposure Quantity due to dietary exposure	PEQ_{di}		µg/bee or µg/bee/day or µg/larva/developmental period	O	
Calculation					
$AR = Q_{prod} \times F_{ai} \times \frac{AREA_{treated}}{AREA_{soil}} \times 10000$					**Equation 12**
$PEQ_{di} = \frac{AR}{1000} \times EF_{di} \times (SV_{po,du} + SV_{ne,du})$					**Equation 1**

For the dietary model for through soil contamination, the only input parameters are the SV parameters for through soil contamination (Table 17). Thus, there is no differentiation between wall or foundation treatment.

Specific input parameters for Tier 1 – through soil contamination

$SV_{po,soil}$ Shortcut values for pollen for through soil contamination apply taking into account that mixed vegetation is growing on the band of soil around the house, see Appendix B. PEC_{pw} parameter for the Tier 1 SV estimation is 1 mg/kg.

$SV_{ne,soil}$ Shortcut values for nectar for through soil contamination apply taking into account that mixed vegetation is growing on the band of soil around the house, see Appendix B. PEC_{pw} parameter for the Tier 1 SV estimation is 1 mg/kg.

Calculations for Tier 1 – through soil contamination

Table 17: Tier 1 calculations for dietary model for through soil contamination

Parameters	Nomenclature	Value	Unit	Origin	Source
Input					
Shortcut value for pollen for through soil contamination	$SV_{po,soil}$		µg/bee or µg/bee/day or µg/larva/developmental period	P	Appendix B
Shortcut value for nectar for through soil contamination	$SV_{ne,soil}$		µg/bee or µg/bee/day or µg/larva/developmental period	P	Appendix B
Output					
Predicted Exposure Quantity due to dietary exposure	PEQ_{di}		µg/bee or µg/bee/day or µg/larva/developmental period	O	
Calculation					
$PEQ_{di} = SV_{po,soil} + SV_{ne,soil}$ **Equation 4**					

5.3.2.3. Tier 2

For the dietary model for above soil contamination in Tier 1, the application rate to the unintentionally contaminated soil area is extrapolated to cover a contaminated soil area of 1 ha. However, within this 1 ha there are also uncontaminated soil areas, as well as non-attractive parts such as terraces or impermeable pathways. Thus, in Tier 2 it is proposed to refine the dietary model for above soil contamination with a so-called contamination factor (F_{cont}). The calculation for the refinement is presented in Table 18.

Specific input parameters for Tier 2 – above soil contamination

N_{houses} Number of houses within 1 ha is set to 16 according to TAB ENV 157 (2022).

F_{cont} Contamination factor. The F_{cont} is a fraction between 0 and 1 and describes the area within 1 ha that is covered with contaminated vegetation attractive to bees. Therefore, it accounts for the dilution of the contaminated area within the total area of 1 ha.

Calculations for Tier 2 – above soil contamination

Table 18: Tier 2 calculations for dietary model for above soil contamination

Parameters	Nomenclature	Value	Unit	Origin	Source

Input					
Quantity of product applied (e.g., to wall, foundation)	Q_{prod}		g/m^2	S	
Fraction of a.s. in the product	F_{ai}			S	
Treated surface (e.g., wall, foundation)	$AREA_{treated}$	125	m^2	D/S	ESD PT18 (2008)
Area of soil that is contaminated	$AREA_{soil}$	26	m^2	D/S	ESD PT18 (2008)
Shortcut value for pollen for above soil contamination – for SUW or DW spraying	$SV_{po,du}$		µg/bee or µg/bee/day or µg/larva/developmental period	P	Appendix B
Shortcut value for nectar for above soil contamination – for SUW or DW spraying	$SV_{ne,du}$		µg/bee or µg/bee/day or µg/larva/developmental period	P	Appendix B
Exposure factor for dietary exposure	EF_{di}	0.1		D	ESD PT18 (2008)
Number of houses on a hectare	N_{house}	16		D	TAB ENV 157 (2022)
Output					
Contamination factor	F_{cont}			O	
Application rate – Tier 2	AR_2		g/ha	O	
Predicted Exposure Quantity due to dietary exposure – Tier 2	$PEQ_{di,2}$		µg/bee or µg/bee/day or µg/larva/developmental period	O	
Intermediate Calculation					
$F_{cont} = AREA_{soil} \times \frac{N_{house}}{10000}$ **Equation 13**					
End Calculation					
$AR_2 = Q_{prod} \times F_{ai} \times \frac{AREA_{treated}}{AREA_{soil}} \times 10000 \times F_{cont}$ **Equation 14**					
$PEQ_{di,2} = \frac{AR_2}{1000} \times EF_{di} \times (SV_{po,du} + SV_{ne,du})$ **Equation 15**					

For the dietary model through soil contamination in Tier 1, the equation for PEQ_{di} derivation is based on a PEC_{pw} of 1 mg/L (Equation 4). However, this is overly conservative for biocidal uses. Therefore, the PEQ_{di} can be refined using the actual calculated PEC_{pw} from the terrestrial risk assessment derived according to Guidance on BPR, Vol. IV, Parts B+C (2017, p. 93, equation 70). Table 19 presents the input and output parameters for Tier 2.

Specific input parameters for Tier 2 – through soil contamination

$PEC_{pw,2}$ Predicted concentration in porewater derived in the terrestrial risk assessment according to Guidance on BPR, Vol. IV, Parts B+C (2017, p. 93, equation 70). It is the result of the fraction of the product applied to the treated surface reaching the soil through deposition (10%), run-off (20%), and wash-off (50%) according to ESD PT 18 (2008).

Calculations for Tier 2 – through soil contamination

Table 19: Tier 2 calculations for dietary model for through soil contamination

Parameters	Nomenclature	Value	Unit	Origin	Source
Input					
Predicted concentration in porewater – Tier 2	$PEC_{pw,2}$		mg/L	S	
Shortcut value for pollen for through soil contamination	$SV_{po,soil}$		µg/bee or µg/bee/day or µg/larva/developmental period	P	Appendix B
Shortcut value for nectar for through soil contamination	$SV_{ne,soil}$		µg/bee or µg/bee/day or µg/larva/developmental period	P	Appendix B
Output					
Predicted Exposure Quantity due to dietary exposure – Tier 2	$PEQ_{di,2}$		µg/bee or µg/bee/day or µg/larva/developmental period	O	
Calculation					
$PEQ_{di,2} = (SV_{po,soil} + SV_{ne,soil}) \times PEC_{pw,2}$				**Equation 10**	

5.3.2.4. Other possible refinements

In general, if unacceptable risks remain after Tier 2, any other parameter used in the exposure calculations could be refined in theory, such as applicants may provide experimental data on the basis of which the exposure factor for contact exposure may be refined. The applicant proposes possible refinement options which need be justified and consulted with the evaluating competent authorities.

For the dietary model for through soil contamination, however, the $PEC_{pw,2}$ could be further refined using FOCUS PEARL (see Appendix C). The resulting PEC_{pw} would be $PEC_{pw,3}$. For more information, see Chapter 5.2.2.3.

5.3.3. Contact exposure model

5.3.3.1. Screening step

The screening step as described in Chapter 5.1.3 could be applied. If unacceptable risk is identified, the risk assessment needs to move to Tier 1. Table 20 presents the input and output parameters for the dietary model for above soil contamination, respectively.

Calculations for Screening step

Table 20: Screening step calculations for contact model

Parameters	Nomenclature	Value	Unit	Origin	Source
Input					
Quantity of product applied to target surface (e.g., wall,	Q_{prod}		g/m^2	S	

foundation)					
Fraction of a.s.	F_{ai}			S	
Treated surface (e.g., wall, foundation)	$AREA_{treated}$	125	m^2	D/S	ESD PT 18 (2008)
Area of soil that is contaminated	$AREA_{soil}$	26	m^2	D/S	ESD PT 18 (2008)
Body surface factor	BSF	0.0114 (HB) 0.0146 (BB) 0.00184 (SB)	dm^2/bee	D	Table 9
Output					
Application rate	AR		g/ha	O	
Predicted Exposure Quantity due to contact exposure	PEQ_{co}		µg/bee	O	
Calculation					
$AR = Q_{prod} \times F_{ai} \times \frac{AREA_{treated}}{AREA_{soil}} \times 10000$ **Equation 12** $PEQ_{co} = AR \times BSF$ **Equation 9**					

5.3.3.2. Tier 1

Table 21 presents the input and the output parameter for the contact model.

Specific input parameters for Tier 1

EF_{co} Exposure factor for contact exposure, value is set to 0.1 which equals the deposition fraction for wall treatment according to ESD PT 18 (2008).

Calculations for Tier 1

Table 21: Tier 1 calculations for contact model

Parameters	Nomenclature	Value	Unit	Origin	Source
Input					
Quantity of product applied to target surface (e.g., wall, foundation)	Q_{prod}		g/m^2	S	
Fraction of a.s.	F_{ai}			S	
Treated surface (e.g., wall, foundation)	$AREA_{treated}$	125	m^2	D/S	
Area of soil that is contaminated	$AREA_{soil}$	26	m^2	D/S	
Exposure factor for contact exposure	EF_{co}	0.1		D	Based on ESD PT 18 (2008).
Body surface factor	BSF	0.0114 (HB) 0.0146 (BB) 0.00184 (SB)	dm^2/bee	D	Table 9
Output					

Application rate	AR		g/ha	O	
Predicted Exposure Quantity due to contact exposure	PEQ_{co}		µg/bee	O	
Calculation					
$AR = Q_{prod} \times F_{ai} \times \frac{AREA_{treated}}{AREA_{soil}} \times 10000$					**Equation 12**
$PEQ_{co} = AR \times EF_{co} \times BSF$					**Equation 7**

5.3.3.3. Tier 2

Table 22 presents the input and the output parameters for Tier 2 of the contact model. The application rate (AR) could be refined in the same way with the contamination factor (F_{cont}) as done for Tier 2 in the dietary model for above soil contamination (see Chapter 5.3.2.3). Applicants may also provide experimental data on the basis of which the exposure factor for contact exposure may be refined. No refinement is possible for the body surface factor.

Specific input parameters for Tier 2

N_{houses} Number of houses within 1 ha is set to 16 according to TAB ENV 157 (2022).
F_{cont} Contamination factor. It represents the actual fraction of contaminated soil within one hectare.

Calculations for Tier 2

Table 22: Tier 2 calculations for contact model

Parameters	Nomenclature	Value	Unit	Origin	Source
Input					
Quantity of product applied to target surface (e.g., wall, foundation)	Q_{prod}		g/m^2	S	
Fraction of a.s.	F_{ai}			S	
Treated surface (e.g., wall, foundation)	$AREA_{treated}$	125	m^2	D/S	
Area of soil that is contaminated	$AREA_{soil}$	26	m^2	D/S	
Exposure factor for contact exposure	EF_{co}	0.1		D	Based on ESD PT18 (2008)
Body surface factor	BSF	0.0114 (HB) 0.0146 (BB) 0.00184 (SB)	dm^2/bee	D	Table 9
Intermediate calculations					
Contamination factor	F_{cont}		g/ha	O	
Output					
Application rate – Tier 2	AR_2		g/ha	O	

Predicted Exposure Quantity due to contact exposure – Tier 2	$PEQ_{co,2}$		ug/bee	O	
Intermediate Calculation					
$F_{cont} = AREA_{soil} \times \frac{N_{house}}{10000}$					**Equation 13**
Calculation					
$AR_2 = Q_{prod} \times F_{ai} \times \frac{AREA_{treated}}{AREA_{soil}} \times 10000 \times F_{cont}$					**Equation 14**
$PEQ_{co,2} = AR_2 \times EF_{co} \times BSF$					**Equation 16**

5.4. Source of exposure – irrigation of private gardens with treated water

5.4.1. Description of source of exposure

PT18 products can directly be applied to small-scale water habitats, e.g., water collectors, water reservoirs, tanks in private areas, and rainwater barrels for mosquito control. Larvicidal products are applied to standing water where mosquitoes could potentially lay eggs. After some time, the same treated water could be used to irrigate private gardens. This use is covered in the TAB entry ENV 205 (October 2022) which describes the emission scenario for the use of treated water for irrigation of private gardens where consequently the active substance is directly released to the soil compartment.

The Treated area scenario is considered the only relevant exposure scenario for the irrigated garden where both the above soil dietary model and contact model are used. This is justified in order to address the exposure due to contamination of plants growing in the garden as a result of shower of water sprinkled or poured over them as well as to address the contamination of plants which may be watered to the root instead. The irrigation of the garden with any systems, e.g., watering can or spraying with hose, is directed downwards. It can be assumed that there is no drift during irrigation and therefore Vegetation margin scenario is not relevant. The Weeds in the treated field and the Plants in the treated area in the next growing season scenarios are covered by the calculation of the Treated area scenario. The plants growing in the garden are considered mixed vegetation which is in flower at the time of application (attractive to bees).

5.4.2. Dietary exposure model

5.4.2.1. Screening step

The screening step as described in Section 5.1.3 could be applied. If unacceptable risk is identified, the risk assessment needs to move to Tier 1. Table 23 presents the input and output parameters for the dietary model for above soil contamination. The input parameters that are needed for the dietary model for above soil contamination are the same as for the standard terrestrial risk assessment for the irrigation scenario (i.e., TAB ENV 205 (2022), Q_{irw}, cir_{1app}, cir_{Napp}). Depending on the use instructions, both irrigation water containing one biocidal application or more have to be assessed. For the risk quantification the higher of two PEQ_{di} values (i.e., either $PEQ_{di,1app}$ or $PEQ_{di,Napp}$) needs to be used.

Specific input parameters for Screening step

Q_{irw} Amount of irrigation water to be used for irrigation of 1 m² garden, value is set to 2.86 L/m²

(according to TAB ENV 205 (2022))

cir_{1app} Concentration of active substance in irrigation water, after one b.p. application, see calculations done for the terrestrial risk assessment (according to TAB ENV 205 (2022))

Napp Number of repeated biocide applications to the water collection container, see calculations done for the terrestrial risk assessment (according to TAB ENV 205 (2022))

cir_{Napp} Concentration of a.s. in irrigation water assuming repeated b.p. application, see calculations done for the terrestrial risk assessment (according to TAB ENV 205 (2022))

n Number of applications per year

B Constant B. Values to be used for downward spray application are applicable for this source of exposure (see Table 10 in Section 5.1.3).

Calculations for Screening step

Table 23: Screening step calculations for dietary model for above soil contamination

Parameters	Nomenclature	Value	Unit	Origin	Source
Input					
Amount of irrigation water to be used for irrigation of 1 m² garden	Q_{irw}	2.86	L/m^2	D	TAB ENV 205 (2022)
Concentration of a.s. in irrigation water, after one b.p. application	cir_{1app}		mg/L	S	
Number of b.p. applications	Napp			S	
Concentration of a.s. in irrigation water assuming repeated b.p. application	cir_{Napp}		mg/L	S	
Number of applications	n			S	
Constant B for SUW spray (wall) or DW spray (foundation)	B		µg/bee or µg/bee/day or µg/larva/ developmental period	P	Table 9
Output					
Application rate after 1 b.p. application	AR_{1app}		g/ha	O	
Application rate after repeated b.p. application	AR_{Napp}		g/ha	O	
Predicted Exposure Quantity due to dietary exposure for 1 b.p. or repeated b.p. applications	$PEQ_{di,1app}$ $PEQ_{di,Napp}$		µg/bee or µg/bee/day or µg/larva/ developmental period	O	
Intermediate calculation					
$AR_{1app} = Q_{irw} \times \frac{cir_{1app}}{1000} \times 10000$					**Equation 17**
$AR_{Napp} = Q_{irw} \times \frac{cir_{Napp}}{1000} \times 10000$					**Equation 18**
End calculation					
$PEQ_{di,1app} = \frac{AR_{1app}}{1000} \times n \times B$					**Equation 19**

$$PEQ_{di,Napp} = \frac{AR_{Napp}}{1000} \times n \times B$$ **Equation 20**

As regards the dietary model for through soil contamination, single default PEQ_{di} values are available, which are independent of application rate, as presented in Table 11. The next step is to compare the PEQ_{di} values calculated by applying Equation 19 and Equation 20 with the PEQ_{di} values reported in Table 11. For each risk case, the highest of the three PEQ_{di} values has to be considered in the risk assessment for the screening step.

5.4.2.2. Tier 1

The Tier 1 assessment is shown in Table 24.

Specific input parameters for Tier 1 – above soil contamination

$SV_{po,du}$ Shortcut values for pollen, downward (DW) spraying taking into account that mixed vegetation is growing in the garden, see Appendix B.

$SV_{ne,du}$ Shortcut values for nectar, downward (DW) spraying taking into account that mixed vegetation is growing in the garden, see Appendix B.

EF_{di} Exposure factor for dietary exposure, value is set to 1 by default for the Treated area scenario.

Calculations for Tier 1 – above soil contamination

Table 24: Tier 1 calculations for dietary model for above soil contamination

Parameters	Nomenclature	Value	Unit	Origin	Source
Input					
Amount of irrigation water to be used for irrigation of 1 m² garden	Q_{irw}	2.86	L/m²	D	TAB ENV 205 (2022)
Concentration of a.s. in irrigation water, after one b.p. application	cir_{1app}		mg/L	S	
Number of b.p. applications	Napp			S	
Concentration of a.s. in irrigation water assuming repeated b.p. application	cir_{Napp}		mg/L	S	
Shortcut value for pollen for above soil contamination – downward spraying	$SV_{po,du}$		µg/bee or µg/bee/day or µg/larva/ developmental period	P	Appendix B
Shortcut value for nectar for above soil contamination – downward spraying	$SV_{ne,du}$		µg/bee or µg/bee/day or µg/larva/ developmental period	P	Appendix B
Exposure factor for dietary exposure	EF_{di}	1		D	EFSA Bee guidance Appendix B
Output					

Application after 1 b.p. application	AR_{1app}		g/ha	O	
Application rate after repeated b.p. application	AR_{Napp}		g/ha		
Predicted Exposure Quantity due to dietary exposure for 1 b.p. or repeated b.p. applications	$PEQ_{di,1app}$ $PEQ_{di,Napp}$		µg/bee or µg/bee/day or µg/larva/ developmental period	O	
Intermediate calculation					
$AR_{1app} = Q_{irw} \times \frac{cir_{1app}}{1000} \times 10000$					**Equation 17**
$AR_{Napp} = Q_{irw} \times \frac{cir_{Napp}}{1000} \times 10000$					**Equation 18**
End calculation					
$PEQ_{di,1app} = \frac{AR_{1app}}{1000} \times EF_{di} \times (SV_{po,du} + SV_{ne,du})$					**Equation 21**
$PEQ_{di,Napp} = \frac{AR_{Napp}}{1000} \times EF_{di} \times (SV_{po,du} + SV_{ne,du})$					**Equation 22**

For the dietary model for through soil contamination, the only input parameters are the SV parameters for through soil contamination (Table 25).

Specific input parameters for Tier 1 – through soil contamination

$SV_{po,soil}$ Shortcut values for pollen, through soil dietary uptake taking into account that mixed vegetation is growing in the garden, see Appendix B. PEC_{pw} parameter for the Tier 1 SV estimation is 1 mg/kg.

$SV_{ne,soil}$ Shortcut values for nectar, through soil dietary uptake taking into account that mixed vegetation is growing in the garden, see Appendix B. PEC_{pw} parameter for the Tier 1 SV estimation is 1 mg/kg.

Calculations for Tier 1 – through soil contamination

Table 25: Tier 1 calculations for dietary model for through soil contamination

Parameters	Nomenclature	Value	Unit	Origin	Source
Input					
Shortcut value for pollen for through soil contamination	$SV_{po,soil}$		µg/bee or µg/bee/day or µg/larva/developmental period	P	Appendix B
Shortcut value for nectar for through soil contamination	$SV_{ne,soil}$		µg/bee or µg/bee/day or µg/larva/developmental period	P	Appendix B
Output					
Predicted Exposure Quantity due to dietary exposure	PEQ_{di}		µg/bee or µg/bee/day or µg/larva/developmental period	O	
Calculation					

$$PEQ_{di} = SV_{po,soil} + SV_{ne,soil}$$ **Equation 4**

5.4.2.3. Tier 2

For the dietary model for above soil contamination in Tier 1, it is assumed that the whole treated hectare consists of attractive garden. However, this is an overly conservative assumption. Thus, a contamination factor (F_{cont}) similar to that applied for the emission scenario for spraying around the house could be used to refine the application rate.

Specific input parameters for Tier 2 – above soil contamination

N_{houses} Number of houses within 1 ha is set to 16 according to TAB ENV 157 (2022).
$AREA_{garden}$ Size of an average European garden is set to 500 m^2 according to ESD PT18 (2008).
F_{attr} Fraction of garden that is attractive to bees is set to 0.8 according to ESD PT 18 (2008).
F_{cont} Contamination factor. It represents the actual fraction of contaminated soil within one hectare.

Calculations for Tier 2 – above soil contamination

Table 26: Tier 2 calculations for dietary model for above soil contamination

Parameters	Nomenclature	Value	Unit	Origin	Source
Input					
Amount of irrigation water to be used for irrigation of 1 m² garden	Q_{irw}	2.86	L/m²	D	TAB ENV 205 (2022)
Concentration of a.s. in irrigation water, after one b.p. application	cir_{1app}		mg/L	S	
Number of b.p. applications	Napp			S	
Concentration of a.s. in irrigation water assuming repeated b.p. application	cir_{Napp}		mg/L	S	
Shortcut value for pollen for above soil contamination – downward spraying	$SV_{po,du}$		µg/bee or µg/bee/day or µg/larva/ developmental period	P	Appendix B
Shortcut value for nectar for above soil contamination – downward spraying	$SV_{ne,du}$		µg/bee or µg/bee/day or µg/larva/ developmental period	P	Appendix B

Exposure factor for dietary exposure	EF_{di}	1		D	EFSA Bee guidance Appendix B
Number of houses per ha	N_{houses}	16		D	TAB ENV 157 (2022)
Average size of private garden	$AREA_{garden}$	500	m^2	D	ESD PT 18 (2008)
Fraction of garden attractive to bees	F_{attr}	0.8		D	ESD PT 18 (2008)
Output					
Contamination factor	F_{cont}			O	
Application rate after 1 b.p. application – Tier 2	$AR_{1app,2}$		g/ha	O	
Application rate after repeated b.p. application – Tier 2	$AR_{Napp,2}$		g/ha		
Predicted Exposure Quantity due to dietary exposure for 1 b.p. or repeated b.p. applications – Tier 2	$PEQ_{di,1app,2}$ $PEQ_{di,Napp,2}$		µg/bee or µg/bee/day or µg/larva/ developmental period	O	
Intermediate calculation					
$F_{cont} = N_{houses} \times AREA_{garden} \times \frac{F_{attr}}{10000}$					**Equation 23**
$AR_{1app,2} = Q_{irw} \times \frac{cir_{1app}}{1000} \times 10000 \times F_{cont}$					**Equation 24**
$AR_{Napp,2} = Q_{irw} \times \frac{cir_{Napp}}{1000} \times 10000 \times F_{cont}$					**Equation 25**
End calculation					
$PEQ_{di,1app,2} = \frac{AR_{1app,2}}{1000} \times EF_{di} \times (SV_{po,du} + SV_{ne,du})$					**Equation 26**
$PEQ_{di,Napp,2} = \frac{AR_{Napp,2}}{1000} \times EF_{di} \times (SV_{po,du} + SV_{ne,du})$					**Equation 27**

For the dietary model through soil contamination in Tier 1, the equation for $PEQ_{j,di}$ derivation is based on a PEC_{pw} of 1 mg/L (Equation 4). However, this is overly conservative for biocidal uses. Therefore, the $PEQ_{j,di}$ can be refined using the actual calculated PEC_{pw} from the terrestrial risk assessment derived according to Guidance on BPR, Vol. IV, Parts B+C (2017, p. 93, equation 70). Table 27 presents the input and output parameters for Tier 2.

Specific input parameters for Tier 2 – through soil contamination

$PEC_{pw,2}$ Predicted concentration in porewater derived in the terrestrial risk assessment according to Guidance on BPR, Vol. IV, Parts B+C (2017, p. 93, equation 70).

Calculations for Tier 2 – through soil contamination

Table 27: Tier 2 calculations for dietary model for through soil contamination

Parameters	Nomenclature	Value	Unit	Origin	Source
Input					
Predicted concentration in porewater – Tier 2	$PEC_{pw,2}$		mg/L	S	
Shortcut value for pollen for through soil contamination	$SV_{po,soil}$		µg/bee or µg/bee/day or µg/larva/developmental period	P	Appendix B
Shortcut value for nectar for through soil contamination	$SV_{ne,soil}$		µg/bee or µg/bee/day or µg/larva/developmental period	P	Appendix B
Output					
Predicted Exposure Quantity due to dietary exposure – Tier 2	$PEQ_{di,2}$		µg/bee or µg/bee/day or µg/larva/developmental period	O	
Calculation					
$PEQ_{di,2} = (SV_{po,soil} + SV_{ne,soil}) \times PEC_{pw,2}$ **Equation 10**					

5.4.2.4. Other possible refinements

No further refinement is possible for the dietary model for above soil contamination.

If needed, the dietary model for through soil contamination could be refined with $PEC_{pw,3}$ as described in the Sections 5.2.2.3 and 5.3.2.4.

5.4.3. Contact exposure model

5.4.3.1. Screening step

The screening step as described in Section 5.1.3 is covered by Tier 1 because the simplified equation for PEQ_{co} (Equation 9) is identical to the equation in Tier 1 because both assume an EF_{co} of 1. Therefore, the assessment can be started with Tier 1.

5.4.3.2. Tier 1

Table 28 presents the input and the output parameter for the contact model.

Specific input parameters for Tier 1

EF_{co} Exposure factor for contact exposure, value is set to 1 by default for Treated area scenario in accordance with EFSA Bee guidance, Appendix B, spraying application.

Calculations for Tier 1

Table 28: Tier 1 calculations for contact exposure

Parameters	Nomenclature	Value	Unit	Origin	Source
Input					
Amount of irrigation water to be used for irrigation of 1 m² garden	Q_{irw}		L/m²	D	TAB ENV 205 (2022)
Concentration of a.s. in irrigation water, after one b.p. application	cir_{1app}		mg/L	S	
Exposure factor for contact exposure	EF_{co}	1		D	EFSA Bee guidance Appendix B
Body surface factor	BSF	0.0114 (HB) 0.0146 (BB) 0.00184 (SB)	dm²/bee	D	Table 9
Output					
Application rate after 1 b.p. application	AR		g/ha	O	
Predicted Exposure Quantity due to contact exposure, after 1 b.p. application	PEQ_{co}		µg/bee	O	
Intermediate calculation					
$AR = Q_{irw} \times \frac{cir_{1app}}{1000} \times 10000$				**Equation 17**	
End calculation					
$PEQ_{co} = AR \times EF_{co} \times BSF$				**Equation 7**	

5.4.3.3. Tier 2

For Tier 2, a refinement of the application rate could be done in line with what is proposed for the dietary model for above soil contamination (Table 29).

Specific input parameters for Tier 2

N_{houses} Number of houses within 1 ha is set to 16 according to TAB ENV 157 (2022).
$AREA_{garden}$ Size of an average European garden is set to 500 m² according to ESD PT18 (2008).
F_{attr} Fraction of garden that is attractive to bees is set to 0.8 according to ESD PT 18 (2008).
F_{cont} Contamination factor. It represents the actual fraction of contaminated soil within one hectare.

Calculations for Tier 2

Table 29: Tier 2 calculations for contact exposure

Parameters	Nomenclature	Value	Unit	Origin	Source
Input					
Amount of irrigation water to be used for irrigation of 1 m² garden	Q_{irw}		L/m²	D	TAB ENV 205 (2022)
Concentration of a.s.	cir_{1app}		mg/L	S	

in irrigation water, after one b.p. application					
Exposure factor for contact exposure	EF_{co}	1		D	EFSA Bee guidance Appendix B
Body surface factor	BSF	0.0114 (HB) 0.0146 (BB) 0.00184 (SB)	dm^2/bee	D	Table 9
Average size of private garden in Europe	$AREA_{garden}$	500	m^2	D	ESD PT 18 (2008)
Fraction of garden attractive to bees	F_{attr}	0.8		D	ESD PT 18 (2008)
Number of houses per ha	N_{houses}	16		D	TAB ENV 157 (2022)
Output					
Contamination factor	F_{cont}			O	
Application rate after 1 b.p. application – Tier 2	AR_2		g/ha	O	
Predicted Exposure Quantity due to contact exposure, after 1 b.p. application – Tier 2	$PEQ_{co,2}$		µg/bee	O	
Intermediate calculation					
$F_{cont} = N_{houses} \times AREA_{garden} \times \frac{F_{attr}}{10000}$ **Equation 23** $AR_2 = Q_{irw} \times \frac{cir_{1app}}{1000} \times 10000 \times F_{cont}$ **Equation 28**					
End calculation					
$PEQ_{co,2} = AR_2 \times EF_{co} \times BSF$ **Equation 29**					

5.5. Source of exposure – large scale spraying of specific species of trees (case A)

5.5.1. Description of source of exposure

This section concerns the assessment of exposure and risk to bees due to application by large scale spraying, in particular application of biocides on specific species of trees by aerial or ground spray against crawling and flying insects, which falls under PT 18 – Outdoor large scale spraying. For further information regarding this use see TAB ENV 248 (2022).

As a result of this application bees may be exposed via overspray, spray drift or soil contamination. Considering that the exposure of bees would not be negligible for this release route, the risk has to be assessed for all relevant pathways.

Case A refers to the application on specific single species of trees (such as oaks, pines, or other woody perennials) likely to take place at forest edges, tree avenues (along the roads in the

cities/countryside) or as solitary tree treatments in parks. These treatments are often performed using a cannon sprayer, unmanned aviation vehicle, or a helicopter. Exposure of bees is assumed due to the consumption of pollen and nectar of the treated trees, of the plants not subject to the treatment but nevertheless affected by the spray deposition on the ground within the treated area, as well as those right next to the treated area. These might consist of flowering plants such as bushes, flowers, grass, berries, etc. At the same time bees may enter in physical contact with the spray containing biocide, or with sprayed plant matrices. Therefore, in order to assess the risk to bees, the following exposure scenarios need to be addressed for this source of exposure:

Treated area,
Weeds in the treated area,
Vegetation margin,
Plants in treated area during the next growing season.

In cases where non-attractive trees are treated, no risk assessment for the Treated area scenario is required.

5.5.2. Dietary exposure model

5.5.2.1. Screening step

The screening step as described in Section 5.1.3 could be applied. If unacceptable risk is identified, the risk assessment needs to move to Tier 1.

Specific input parameters for Screening step

AR — The application rate of active substance in mass units per hectare of treated area may be estimated by assuming that treated trees with given crown diameter are growing densely next to each other on a hectare and considering the application rate of active substance in kg/tree or similar.

B — Where the application is carried out by helicopter, downwards spraying values for constant B are recommended. Where the application is carried out by a cannon sprayer, sideward spraying values for constant B are recommended. Where spraying technology for biocides applications is not matching with agricultural spraying techniques, or where details of the spraying technique would not be available during the assessment, values for constant B for sideward/upward spray application are applicable as a worst-case choice. See Table 10.

Calculations for Screening step

Table 30: Screening step calculations for dietary exposure for above soil contamination

Parameters	Nomenclature	Value	Unit	Origin	Source
Input					
Application rate	AR		g/ha	S	same as $Q_{a.i.}$ in TAB ENV 248 (2022)
Number of applications	n		[-]	S	
Constant B	B		µg/bee or µg/bee/day or µg/larva/developmental period	P	Table 10
Output					
Predicted Exposure Quantity due to dietary exposure	PEQ_{di}		µg/bee or µg/bee/day or µg/larva/developmental period	O	

Calculation	
$PEQdi = \frac{AR}{1000} \times n \times B$	**Equation 8**

As regards the through soil contamination, single default PEQ_{di} values are available, which are independent of application rate, as presented Table 11. The next step is to compare the PEQ_{di} values calculated by applying Equation 8 with the PEQ_{di} values reported in Table 11. For each risk case, the highest of the two PEQ_{di} values has to be considered in the risk assessment for the screening step.

5.5.2.2. Tier 1

Generally, in Tier 1 it is assumed that the biocidal application is performed on trees in spring or summer and the treated plants are attractive to bees (worst case assumption) and while they are flowering. In case of a specific tree species is treated and where the tree species is not attractive to bees for pollen and/or nectar, Treated area may not need to be assessed (if tree is not attractive for pollen, nor nectar) or some shortcut values may not need to used (if tree is not attractive for either pollen or nectar). For more information on attractiveness see EFSA Bee guidance, Appendix I and also Appendix A of this ECHA Bee Guidance.

On the basis of the above, assessment of exposure scenarios Treated area, Weeds in the treated area and Vegetation margin is based on the dietary model for above soil contamination and the respective defaults from the EFSA Bee guidance and TAB ENV 248 (2022). Through soil contamination is considered covered by the assessment of the above soil exposure for these exposure scenarios. The dietary model for through soil contamination is relevant for the Plants in treated area during the next growing season scenario.

Specific input parameters for Tier 1 – above soil contamination

EF_{di} For Treated area scenario – value of 1 for spray application from the EFSA Bee guidance, Appendix B is applicable.
For Weeds in the treated area scenario – the most relevant values are those of surrogate crops such as "Olives (evergreen)"[6] or "Pome/stone fruits"[7] from the EFSA Bee guidance, Appendix B. The choice depends on the similarity in the morphology/growth pattern of the treated trees compared to the species considered covered by these surrogate crop categories of EFSA. EF_{di} for this scenario depends on the growth stage of the treated plant (linked with interception by the treated plant). The worst case EF_{di} value for the two surrogate crops in case of application by spraying of 0.5 is considered relevant for this source of exposure (from "Pome/stone fruits" crop category at the time of sprouting/bud development. Beginning of dormancy growth stage of this surrogate crop is considered not relevant for biocides.). The EF_{di} value of 0.3, originating from surrogate crop "Olives (evergreen)", may be considered for coniferous trees.
For Vegetation margin scenario – in accordance with TAB ENV 248 (2022) the highest drift

[6] The crop category "Olives (evergreen)" covers Olives / *Populus sp. Salix sp. / Acer spp., Alnus spp., Betula spp., Castanea spp., Crataegus spp., Cornus spp., Fraxinus spp., Juglans spp., Populus spp., Prunus spp., Salix spp., Sorbus spp., Quercus spp., Tilia spp., Ulmus spp., Carpinus betulus, Corylus avellana, Fagus sylvatica, Malus sylvestris* and *Pyrus pyraster / Abies spp., Cupressus spp., Picea spp., Pinus spp., Cedrus spp.* and *Larix spp., Juniperus communis, Pseudotsuga menziesii* and *Taxus baccata/* this group includes e.g. *Abies spp.*, *Cedrus spp.*, *Chamaecyparis spp.*, *Cupressaceae spp.*, *Ephedra spp.*, *Juniperus spp.*, *Larix spp.*, *Picea spp., Pinus spp., Pseudotsuga spp., Thuja spp.* and *Tsuga spp*. Species such as *Araucaria araucana* and *Taxus baccata* are also relevant.

[7] The crop category "Pome/stone fruits" covers banana / fig / chestnut, walnut, pinenut, pistachio / this group includes ornamental broad-leaved trees of e.g. *Acacia spp., Acer spp., Betula spp., Erica spp., Fagus spp., Ilex spp., Hibiscus spp., Hydrangea spp., Populus spp., Quercus spp., Tilia spp.*, ornamental shrubs of e.g. *Buxus spp., Crataegus spp., Ligustrum spp., Rosa spp., Viburnum spp., Rhododendron spp.*, and woody climbing plants such as *Bougainvillea spectabilis, Hedera helix, Jasminum nudiflorum*. / this group includes trees and shrubs such as palms and bamboos. *Cycads spp., Ginkgo spp.*, and *Gnetum spp.* are also included. / ornamental broad-leaved trees, shrubs, and climbing plants ornamental conifers ornamental woody monocotyledonous plants / pear, apple / peach, plum, almond, cherry, apricot

value derived for "trees (early stage, > 2m)" of 38.09 % agreed as a general default in case of assessment of biocides is considered relevant also as a basis for EF_{di} for this exposure scenario. TAB ENV 248 (2022) provides also drift values for different application techniques and field of uses in treatment against the oak processionary moth, which may be used when relevant.

$SV_{po,du}$; $SV_{ne,du}$ The biocides application as a worst case assumption is considered to take place during flowering of the treated tree. Where the application is carried out by helicopter, downwards spraying SV are recommended. Where the application is carried out by a cannon sprayer, sideward spraying SV are recommended. Where spraying technology for biocides applications is not matching with agricultural spraying techniques, or where details of the spraying technique would not be available during the assessment, a worst case set of SVs independent of spraying technique (set up for biocides) should be used. SVs are presented in Appendix B (based on EFSA Bee guidance Appendix B). Selection of the SV is made on the basis of the number of applications during the year and the interval between multiple applications.

Since Weeds in treated area and Plants in the Vegetation margin are considered as habitats with mixed vegetation, sugar content of 30 % is applicable (see Table 7). Consequently, SVs for nectar for these scenarios should be calculated as follows: multiply the original $SV_{ne,du}$ by 1/3 for SB groups; by ½ for HB and BB groups.

Where treated trees are attractive for one matrix only e. g. pollen (oaks), SVs for the matrix which is not relevant (i. e. nectar in this case) are considered 0 in the Treated area scenario.

Calculations for Tier 1 – above soil contamination

Table 31: Tier 1 calculations for dietary model for above soil contamination (Treated area, Weeds in the treated area, Vegetation margin)

Parameters	Nomenclature	Value	Unit	Origin	Source
Input					
Application rate	AR		g/ha	S	same as $Q_{a.i.}$ in TAB ENV 248 (2022)
Exposure factor for dietary exposure	EF_{di}		[-]		
Treated area		1		D	EFSA Bee guidance, Appendix B
Weeds in the treated area		0.5/0.3		D	EFSA Bee guidance, Appendix B
Vegetation margin		0.381		D/S	TAB ENV 248 (2022)
Shortcut value for pollen for during flowering situations	$SV_{po,du}$		µg/bee or µg/bee/day or µg/larva/developmental period	P	Appendix B
Shortcut value for nectar for during flowering situations	$SV_{ne,du}$		µg/bee or µg/bee/day or µg/larva/developmental period	P	Appendix B Multiply original SV_{ne} for Weeds in the treated area and Vegetation margin scenarios, with 1/3 for SB; by ½ for HB and BB
Output					
Predicted Exposure Quantity due to	PEQ_{di}		µg/bee or µg/bee/day or µg/larva/developmen	O	

dietary exposure			tal period		
Calculation					
$PEQ_{di} = \frac{AR}{1000} \times EF_{di} \times (SV_{po,du} + SV_{ne,du})$ **Equation 1**					

Specific input parameters for Tier 1 – through soil contamination

$SV_{po,soil}$ Shortcut values are based on a PEC_{pw} of 1 mg/kg, which can be considered as an extreme worst-case for biocides. SVs for pollen from EFSA model "through soil contamination" apply, see Appendix B.

$SV_{ne,soil}$ The shortcut values are based on a PEC_{pw} of 1 mg/kg, which can be considered as an extreme worst-case for biocides. SVs for nectar from EFSA model "through soil contamination" apply, see Appendix B.

Calculations for Tier 1 – through soil contamination

Table 32: Tier 1 calculations for dietary model for through soil contamination (Plants in treated area during the next growing season)

Parameters	Nomenclature	Value	Unit	Origin	Source
Input					
Shortcut value for pollen for through soil contamination	$SV_{po,soil}$		µg/bee or µg/bee/day or µg/larva/developmental period	P	Appendix B
Shortcut value for nectar for through soil contamination	$SV_{ne,soil}$		µg/bee or µg/bee/day or µg/larva/developmental period	P	Appendix B
Output					
Predicted Exposure Quantity due to dietary exposure	PEQ_{di}		µg/bee or µg/bee/day or µg/larva/developmental period	O	
Calculation					
$PEQ_{di} = SV_{po,soil} + SV_{ne,soil}$ **Equation 4**					

5.5.2.3. Tier 2

Treated area, Weeds in the treated area, Vegetation margin

In Tier 2, PEQ_{di} can be refined by changing the parameters of the calculations in Tier 1 presented above.

Specific input parameters for Tier 2 – above soil contamination

AR Applicants may propose typical areas relevant for the treatment of single trees, tree avenues, forest edges, where different spacing between treated trees may be assumed. See section 5.3 (in relation to contamination factor (F_{cont})). Furthermore, reduced AR may be considered provided that it is confirmed by efficacy data. See Table 31.

SV Since SVs are a function of the number of applications during the year and the interval between multiple applications, lower number of applications per year and/or larger interval between applications may be considered as a form of refinement, which will result in smaller SVs. Such change needs to be confirmed by efficacy data.

EF_{di} For Vegetation margin scenario – applicants may provide experimental data on the basis of which the default drift value described above may be refined. See Table 31.

For other options for refinement, see EFSA Bee guidance sections 5.4 and 5.5. Proposals for refinements need be justified and consulted with the evaluating competent authorities.

Plants in treated area during the next growing season

Refer to Section 5.2.2.2. For the large-scale spraying case A nevertheless, considerations described below apply.

Specific input parameters for Tier 2 – through soil contamination

$PEC_{pw,2}$ Predicted environmental concentration in porewater calculated from PEC_{soil} initial (in case of multiple applications after last application) after ten consecutive years of application, taking degradation into account in line with TAB ENV 248 (2022) following equation 70 of Biocides Guidance Volume IV Part B and C. Time-weighted average (TWA) concentration in soil over 180 days after the last application after 10 consecutive years of application may be considered as a second Tier (TAB ENV 237 (2022)).

5.5.2.4. Tier 3

Plants in treated area during the next growing season

In Tier 3, the same calculation as in Tier 1 is conducted but the shortcut values are re-calculated based on a product specific PEC_{pw}. The PEC_{pw} in Tier 2, which is calculated from PEC_{soil} in the biocides exposure assessment and is considered as a conservative value, can be refined by the modelling tool FOCUS PEARL. For more information, see Section 5.2.2.3.

5.5.3. Contact exposure model

5.5.3.1. Screening step

The screening step as described in Section 5.1.3 could be applied. If unacceptable risk is identified, the risk assessment needs to move to Tier 1.

Specific input parameters for Screening step

AR See section 5.5.2.1

Calculations for Screening step

Table 33: Screening step calculations for contact exposure

Parameters	Nomenclature	Value	Unit	Origin	Source
Input					
Application rate	AR		g/ha	S	same as $Q_{a.i.}$ in TAB ENV 248 (2022)
Body surface factor	BSF	0.0114 (HB) 0.0146 (BB) 0.00184 (SB)	dm^2/bee	P	Table 9
Output					
Predicted Exposure Quantity for contact exposure	PEQ_{co}		µg/bee	O	
Calculation					
$PEQ_{co} = AR \times BSF$ **Equation 9**					

5.5.3.2. Tier 1

Generic considerations described under Section 5.5.2.2 Tier 1 apply.
Contact exposure is relevant for exposure scenarios Treated area, Weeds in the treated area and Vegetation margin and Tier 1 is based on the respective defaults from the EFSA Bee guidance and TAB ENV 248 (2022). Contact exposure is not relevant for the Plants in treated area during the next growing season exposure scenario and therefore PEQ_{co} value for this scenario is equal 0 in the combined risk assessment.

Specific input parameters for Tier 1

AR — see section 5.5.2.2 Tier 1

EF_{co} — For Treated area scenario – worst case value of 1 associated with the flowering stage of the treated trees in case of spray application from the EFSA Bee guidance, Appendix B is applied.
For Weeds in the treated area scenario – the most relevant values are those of surrogate crops such as "Olives (evergreen)"[6] or "Pome/stone fruits"[7] from the EFSA Bee guidance, Appendix B. The choice depends on the similarity in the morphology/growth pattern of the treated trees compared to the species considered covered by these surrogate crop categories of EFSA. EF_{co} for this scenario depends on the growth stage of the treated plant (linked with interception by the treated plant). The worst case EF_{co} value for the two surrogate crops in case of application by spraying of 0.5 is considered relevant for this source of exposure (from "Pome/stone fruits" crop category at the time of sprouting/bud development. Beginning of dormancy growth stage of this surrogate crop is considered not relevant.). The EF_{co} value of 0.3, originating from surrogate crop "Olives (evergreen)", may be considered for coniferous trees.
For Vegetation margin scenario – in accordance with TAB ENV 248 (2022) the highest drift value derived for "trees (early stage, > 2m)" of 38.09 % agreed as a general default in case of assessment of biocides is considered relevant also as a basis for EF_{co} for this exposure scenario. TAB ENV 248 (2022) provides also drift values for different application techniques and field of uses in treatment against the oak processionary moth, which may be used when relevant.

Calculations for Tier 1

Table 34: Tier 1 calculations for contact exposure (Treated area, Weeds in the treated area, Vegetation margin)

Parameters	Nomenclature	Value	Unit	Origin	Source
Input					
Application rate	AR		g/ha	S	same as $Q_{a.i.}$ in TAB ENV 248 (2022)
Exposure factor for dietary exposure	EF_{co}		[-]		
Treated area		1		D	EFSA Bee guidance, Appendix B
Weeds in the treated area		0.5/0.3		D	Based on EFSA Bee guidance, Appendix B
Vegetation margin		0.381		D/S	TAB ENV 248 (2022)
Body surface factor	BSF	0.0114 (HB) 0.0146 (BB) 0.00184 (SB)	dm^2/bee	P	Table 9
Output					
Predicted Exposure Quantity for	PEQ_{co}		µg/bee	O	

contact exposure					
Calculation					
$PEQ_{co} = AR \times EF_{co} \times BSF$				**Equation 7**	

5.5.3.3. Tier 2

In Tier 2, PEQ_{co} can be refined by changing the parameters of the calculations in Tier 1 presented above.

Specific input parameters for Tier 2

AR — Applicants may propose typical areas relevant for treatment of single trees, tree avenues, forest edges, where different spacing between treated trees may be assumed. See section 5.3 (in relation to contamination factor). Furthermore, reduced application rate may be considered provided that it is confirmed by efficacy data. See Table 34.

EF_{co} — For Vegetation margin scenario – applicants may provide experimental data on the basis of which the default drift value described above may be refined. See Table 34.

5.6. Source of exposure – large scale spraying of mixed species of trees and bushes (case B)

5.6.1. Description of source of exposure

This section concerns the assessment of exposure and risk to bees due to application by large scale spraying, in particular application of biocides on mixed species of trees or shrubs by ground spray against crawling and flying insects (e.g., for mosquito control), which falls under PT 18 – Outdoor large scale spraying. For further information regarding this use see TAB ENV 248 (2022).[8]

As a result of this application, bees may be exposed via overspray, spray drift, or soil contamination. Considering that the exposure of bees would not be negligible for this release route, the risk has to be assessed for all relevant pathways.

Case B refers to the application on mixed species of trees and bushes (woody perennials) likely to take place in amenity areas. Parallels may be drawn with a surrogate crop called "ornamentals", distinguished by the EFSA Bee guidance, which refers to a diverse group of plants, grown in a variety of ways, which can vary from small herbaceous plants to large ornamentals trees. Exposure of bees is assumed due to the consumption of pollen and nectar of the treated plants, of the plants not subject to the treatment but nevertheless affected by the spray deposition on the ground within the treated area, as well as those right next to the treated area. These might consist of flowering plants such as bushes, flowers, grass, berries, etc. At the same time bees may enter in physical contact with the spray containing biocide or with sprayed plant matrices. Therefore, in order to assess the risk to bees, the following exposure scenarios need to be addressed for this source of exposure:

Treated area,
Weeds in the treated area,
Vegetation margin,
Plants in treated area during the next growing season.

Since mixed plants are being treated, as a worst-case assumption these are considered attractive

[8] Ultra low volume (ULV) spraying is out of scope of TAB ENV 248 (2022) and therefore also this guidance.

to bees for both pollen and nectar.

5.6.2. Dietary exposure model

5.6.2.1. Screening step

The screening step as described in Section 5.1.3 could be applied. If unacceptable risk is identified, the risk assessment needs to move to Tier 1.

Specific input parameters for Screening step

AR — The application rate of active substance in mass units per hectare of treated area may be estimated by assuming that treated plants are growing densely next to each other on a hectare and considering application rate of active in kg/model plant or similar.

For further input parameters and calculations, see Section 5.5.2.1.

5.6.2.2. Tier 1

In Tier 1 it is assumed that the biocidal application is performed on plants in spring or summer and the treated plants are attractive to bees (worst case assumption) and while they are flowering.

On the basis of the above, assessment of exposure scenarios Treated area, Weeds in the treated area, and Vegetation margin is based on the dietary model for above soil contamination and the respective defaults from the EFSA Bee guidance and TAB ENV 248 (2022). Through soil contamination is considered covered by the assessment of the above soil exposure for these exposure scenarios. The dietary model for through soil contamination is relevant for the Plants in treated area during the next growing season scenario.

Specific input parameters for Tier 1 – above soil contamination

EF_{di} — For Treated area scenario – value if 1 for spray application from the EFSA Bee guidance, Appendix B is applicable.
For Weeds in the treated area scenario – the most relevant value is 1 as also suggested for the surrogate crop "Ornamentals" from the EFSA Bee guidance, Appendix B which does not have its own deposition categories (i.e., the value for the EF_{di} equals always 1). It is a worst-case surrogate crop applicable in cases when diverse group of plants are treated.
For Vegetation margin scenario – assuming that the height of treated plants may vary up to the height of forest trees, in accordance with TAB ENV 248 (2022) the highest drift value derived for "trees (early stage, > 2m)" of 38.09 % agreed as a general default in case of assessment of biocides, is considered relevant also as a basis for EF_{di} for this exposure scenario. TAB ENV 248 (2022) provides also drift values for different application techniques and field of uses in treatment against the oak processionary moth, which may be used when relevant.

$SV_{po,du}$; $SV_{ne,du}$ — See Section 5.5.2.2 regarding the selection of SVs.
In contrast to Case A, in Case B the treatment is applied to unknown mixed species of trees and bushes. Consequently, the Treated area is considered as a habitat with mixed vegetation and a sugar content of 30% (the same refers to Weeds in treated area and Vegetation margin). Consequently, SVs for nectar for all these exposure scenarios should be calculated as follows: multiply the original SV (nectar) by 1/3 for SB groups; by ½ for HB and BB groups.

Calculations for Tier 1 – above soil contamination

Table 35: Tier 1 calculations for dietary model for above soil contamination (Treated area, Weeds in the treated area, Vegetation margin)

Parameters	Nomenclature	Value	Unit	Origin	Source
Input					

Application rate	AR		g/ha	S	same as $Q_{a.i.}$ in TAB ENV 248 (2022)
Exposure factor for dietary exposure Treated area Weeds in the treated area Vegetation margin	EF_{di}	 1 1 0.381	[-]	 D D D/S	 EFSA Bee guidance, Appendix B EFSA Bee guidance, Appendix B TAB ENV 248 (2022)
Shortcut value for pollen for during flowering situations	$SV_{po,du}$		µg/bee or µg/bee/day or µg/larva/developmental period	P	Appendix B
Shortcut value for nectar for during flowering situations	$SV_{ne,du}$		µg/bee or µg/bee/day or µg/larva/developmental period	P	Appendix B Multiply original SV_{ne} by 1/3 for SB; by ½ for HB and BB
Output					
Predicted Exposure Quantity due to dietary exposure	PEQ_{di}		µg/bee or µg/bee/day or µg/larva/developmental period	O	
Calculation					
$PEQ_{di} = \frac{AR}{1000} \times EF_{di} \times \left(SV_{po,du} + SV_{ne,du}\right)$					**Equation 1**

Provided that the above assumptions are maintained, Treated area scenario and Weeds in the treated area scenario are identical.

Specific input parameters for Tier 1 – though soil contamination

$SV_{po,soil}$; $SV_{ne,soil}$ see section 5.5.2.2 Tier 1

In contrast to Case A, in Case B the treatment is applied to unknown mixed species of trees and bushes. Consequently, the Plants in treated area during the next growing season scenario are considered as a habitat with mixed vegetation and a sugar content of 30%. Consequently, SVs for nectar for all exposure scenarios should be calculated as follows: multiply the original SV (nectar) by 1/3 for SB groups; by ½ for HB and BB groups.

Calculations for Tier 1 – through soil contamination

Table 36: Tier 1 calculations for dietary model for through soil contamination (Plants in treated area during the next growing season)

Parameters	Nomenclature	Value	Unit	Origin	Source
Input					
Shortcut value for pollen for through soil contamination	$SV_{po,soil}$		µg/bee or µg/bee/day or µg/larva/developmental period	P	Appendix B
Shortcut value for nectar for through soil contamination	$SV_{ne,soil}$		µg/bee or µg/bee/day or µg/larva/developmental period	P	Appendix B with adjusted Svne, as explained above

Output					
Predicted Exposure Quantity due to dietary exposure	PEQ_{di}		µg/bee or µg /bee/day or µg/larva/developmental period	O	
Calculation					
$PEQ_{di} = SV_{po,\text{soil}} + SV_{ne,soil}$				**Equation 4**	

5.6.2.3. Tier 2

Treated area, Weeds in the treated area, Vegetation margin

In Tier 2, PEQ_{di} can be refined by changing the parameters of the calculations in Tier 1 presented above.

Specific input parameters for Tier 2 – above soil contamination

AR — Applicants may propose typical areas where treatment of selected spots (among those shrubs and trees) is relevant. In such case, ratio of treated areas and untreated areas covered with vegetation may need to be assumed. See Section 5.3 (in relation to contamination factor). Furthermore, reduced application rate may be considered provided that it is confirmed by efficacy data. See Table 35.

SV — Since SVs are a function of the number of applications during the year and the interval between multiple applications, lower number of applications per year and/or larger interval between applications may be considered as a form of refinement, which will result in a smaller SV. Such change needs to be confirmed by efficacy data.

EF_{di} — For Vegetation margin scenario – applicants may provide experimental data on the basis of which the default drift value described above may be refined. See Table 35

For other options for refinement, see EFSA Bee guidance Sections 5.4 and 5.5. Proposals for refinements need be justified and consulted with the evaluating competent authorities.

Plants in treated area during the next growing season

Refer to Section 5.2.2.2. For the large scale spraying case B nevertheless, considerations described below apply.

Specific input parameters for Tier 2 – through soil contamination

$PEC_{pw,2}$ — Predicted environmental concentration in porewater calculated from PEC_{soil} initial (in case of multiple applications after last application) after ten consecutive years of application, taking degradation into account in line with TAB ENV 248 (2022) following equation 70 of Biocides Guidance Volume IV Part B + C. Time-weighted average (TWA) concentration in soil over 180 days after the last application after 10 consecutive years of application may be considered as a second Tier (TAB ENV 237 (2022)).

5.6.2.4. Tier 3

Plants in treated area during the next growing season

In Tier 3, the same calculation as in Tier 1 is conducted but the shortcut values are re-calculated based on a product specific PEC_{pw}. The PEC_{pw} in Tier 2, which is calculated from PEC_{soil} in the biocides exposure assessment and is considered as a conservative value, can be refined by the modelling tool FOCUS PEARL. For more information, see Section 5.2.2.3.

5.6.3. Contact exposure model

5.6.3.1. Screening step

The screening step as described in Section 5.1.3 could be applied. If unacceptable risk is identified, the risk assessment needs to move to Tier 1. For more specific information, see Section 5.5.3.1.

Specific input parameters for Screening step

AR See Section 5.6.2.1

For calculations, see Table 33.

5.6.3.2. Tier 1

Generic considerations described under Section 5.6.2.2 apply.
Contact exposure is relevant for exposure scenarios Treated area, Weeds in the treated area and Vegetation margin, and Tier 1 is based on the respective defaults from the EFSA Bee guidance and TAB ENV 248 (2022). Contact exposure is not relevant for the Plants in treated area during the next growing season exposure scenario and therefore PEQ_{co} value for this scenario is equal to 0 in the combined risk assessment.

Specific input parameters for Tier 1

AR See Section 5.6.2.2 Tier 1

EF_{co} For Treated area scenario – value of 1 relevant for the flowering stage of the treated plants in case of spray application from the EFSA Bee guidance, Appendix B is applied.
For Weeds in the treated area scenario – the most relevant value is 1 as also suggested for the surrogate crop "Ornamentals" from the EFSA Bee guidance, Appendix B which does not have its own deposition categories (i.e., the value for the EF_{co} equals always 1). It is a worst-case surrogate crop applicable in cases when a diverse group of plants is treated.
For Vegetation margin scenario – assuming that the height of treated plants may vary up to the height of forest trees, in accordance with TAB ENV 248 (2022) the highest drift value derived for "trees (early stage, > 2m)" of 38.09 % agreed as a general default in case of assessment of biocides, is considered relevant also as a basis for EF_{di} for this exposure scenario. TAB ENV 248 (2022) provides also drift values for different application techniques and field of uses in treatment against the oak processionary moth, which may be used when relevant.

Calculations for Tier 1

Table 37: Tier 1 calculations for contact exposure (Treated area, Weeds in the treated area, Vegetation margin)

Parameters	Nomenclature	Value	Unit	Origin	Source
Input					
Application rate	AR		g/ha	S	same as $Q_{a.i.}$ in TAB ENV 248 (2022)
Exposure factor for dietary exposure	EF_{co}		[-]		
Treated area		1		D	EFSA Bee guidance, Appendix B
Weeds in the treated area		1		D	EFSA Bee guidance, Appendix B
Vegetation margin		0.381		D/S	TAB ENV 248 (2022)

Body surface factor	BSF	0.0114 (HB) 0.0146 (BB) 0.00184 (SB)	dm^2/bee	P	Table 9
Output					
Predicted Exposure Quantity for contact exposure	PEQ_{co}		µg/bee	O	
Calculation					
$PEQ_{co} = AR \times EF_{co} \times BSF$ **Equation 7**					

5.6.3.3. Tier 2

In Tier 2, PEQ can be refined by changing the parameters of the calculations in Tier 1 presented above.

Specific input parameters for Tier 2

AR — Applicants may propose typical areas where treatment of selected spots (among those shrubs and trees) is relevant. In such case, ratio of treated areas and untreated areas covered with vegetation may need to be assumed. See Section 5.3 (in relation to contamination factor). Furthermore, reduced application rate may be considered provided that it is confirmed by efficacy data. See Table 37.

EF_{co} — For Vegetation margin scenario – applicants may provide experimental data on the basis of which the default drift value described above may be refined. See Table 37.

5.7. Source of exposure – large scale spraying of natural water bodies (case C)

5.7.1. Description of source of exposure

This section concerns the assessment of exposure and risk to bees due to application by large scale spraying, in particular application of biocides on natural water bodies. In accordance with ESD PT 18 for household and professional uses (2008), spray treatment of natural water bodies to control mosquito larvae may be operated on large scale by fixed-wing aircraft or helicopters. Depending on the structure of the landscape, mosquito control may also be performed from the edge of a water body, using standard truck-mounted mosquito abatement equipment. The development of mosquito larvae can also occur in storm water treatment devices. In California, mosquito larvicides are applied using hand-held equipment at small sites and with backpack or truck-mounted high-pressure sprayers at large sites (Metzger 2004). ESD PT 18 (2008) recommends adapting the scenarios for exposure assessment of plant protection products for crops grown in water (e.g., rice) in order to assess this biocidal application (European Commission, 2003b, so called MED-RICE scenario).

As a result of this application, bees may be exposed via spray drift. Considering that the exposure of bees would not be negligible for this release route, the risk has to be assessed for this pathway.

Case C refers to the application on natural water bodies which may result in deposits to a vegetation margin (banks) around the treated water bodies. Exposure of bees is assumed due

to the consumption of pollen and nectar of the plants outside the treated area[9]. These might consist of flowering plants such as trees, bushes, flowers, grass or berries growing right next to the treated water body. At the same time bees may enter in physical contact with the spray containing biocide or with sprayed plant matrices. Therefore, in order to assess the risk to bees, the following exposure scenario need to be addressed for this source of exposure:

Vegetation margin.

5.7.2. Dietary exposure model

5.7.2.1. Screening step

See Section 5.5.2.1 Screening step.

Specific input parameters for Screening step

AR — The application rate of active substance in mass units per hectare of treated water body surface is a necessary input into the exposure calculations. AR may need to be calculated from target concentration in the water body and based on the treated water body dimensions. In addition, for the running water, water volume flow per hour (i.e., width (m) x depth (m) x flow rate (m/hour)) needs to be considered.

For further input parameters and calculations, see Section 5.5.2.1. As through soil contamination is not relevant for this source of exposure, PEQ_{di} values presented in Table 11 are not relevant.

5.7.2.2. Tier 1

In Tier 1 it is assumed that the biocidal application is performed on water bodies during spring and summer and the banks of the treated water bodies are covered by a variety of unknown plants which may flower at the time of application. Consequently, the assessment of exposure scenario Vegetation margin is based on the dietary model for above soil contamination and the respective defaults from the EFSA Bee guidance and TAB ENV 248 (2022). Considering there is no agreed emission scenario for this type of use, the respective defaults proposed in this guidance may be replaced by different ones when such an emission scenario will become available. Through soil contamination is considered covered by the assessment of the above soil exposure for this exposure scenario.

Specific input parameters for Tier 1 – above soil contamination

EF_{di} — For Vegetation margin scenario – as a worst case, drift value derived for "trees (early stage, > 2m)" of 38.09 % agreed as a general default in case of assessment of biocides (ENV 248), is considered relevant also as a basis for EF_{di}[10]. Nevertheless, in case of handheld devices another drift value may be accepted.

$SV_{po,du}$; $SV_{ne,du}$ See Section 5.5.2.2 regarding the selection of SVs.
Since plants on the banks of treated water bodies are considered as a habitat with mixed vegetation, sugar content of 30 % is applicable for Vegetation margin scenario, see Table 7. Consequently, SVs for nectar for this scenario should be calculated as follows: multiply the original SV (nectar) by 1/3 for SB groups; by ½ for HB and BB groups.

Calculations for Tier 1

Table 38: Tier 1 calculations for dietary model for above soil contamination (Vegetation margin)

Parameters	Nomenclature	Value	Unit	Origin	Source
Input					

[9] In accordance with EFSA Guidance, exposure from contaminated water is not included in the risk assessment of bees.
[10] FOCUS (2001) suggests aerial drift loadings 33.2% for PPP.

Application rate	AR		g/ha	S	
Exposure factor for dietary exposure Vegetation margin	EF_{di}	0.381	[-]	D/S	TAB ENV 248 (2022)
Shortcut value for pollen for during flowering situations	$SV_{po,du}$		µg/bee or µg/bee/day or µg/larva/developmental period	P	Appendix B
Shortcut value for nectar for during flowering situations	$SV_{ne,du}$		µg/bee or µg/bee/day or µg/larva/developmental period	P	Appendix B Multiply original SV_{ne} by 1/3 for SB; by ½ for HB and BB
Output					
Predicted Exposure Quantity due to dietary exposure	PEQ_{di}		µg/bee or µg/bee/day or µg/larva/developmental period	O	
Calculation					
$PEQ_{di} = \frac{AR}{1000} \times EF_{di} \times \left(SV_{po,du} + SV_{ne,du}\right)$					**Equation 1**

5.7.2.3. Tier 2

In Tier 2, PEQ_{di} can be refined by changing the parameters of the calculations in Tier 1 presented above.

Specific input parameters for Tier 2

AR — Reduced application rate may be considered provided that it is confirmed by efficacy data. See Table 38.

SV — Since SVs are a function of the number of applications during the year and the interval between multiple applications, lower number of applications per year and/or larger interval between applications may be considered as a form of refinement, which will result in smaller SVs. Such change needs to be confirmed by efficacy data.

EF_{di} — For Vegetation margin scenario – applicants may provide experimental data on the basis of which the default drift value described above may be refined. See Table 38.

For other options for refinement, see EFSA Bee guidance Sections 5.4 and 5.5. Proposals for refinements need be justified and consulted with the evaluating competent authorities.

5.7.3. Contact exposure model

5.7.3.1. Screening step

The screening step as described in Section 5.1.3 could be applied. If unacceptable risk is identified, the risk assessment needs to move to Tier 1.

Specific input parameters for Screening step

AR — See Section 5.7.2.1

For calculations see Table 33.

5.7.3.2. Tier 1

Generic considerations described under Section 5.7.2.2.

Contact exposure is relevant for the exposure scenario Vegetation margin, and Tier I is based on the respective defaults from the EFSA Bee guidance and TAB ENV 248 (2022).

Specific input parameters for Tier 1

AR — See Section 5.7.2.2

EF_{co} — For Vegetation margin scenario – as a worst case, drift value derived for "trees (early stage, > 2m)" of38.09 % agreed as a general default in case of assessment of biocides (TAB ENV 248 (2022)), is considered relevant also as a basis for EF_{co}[10]. Nevertheless, in case of handheld devices another drift value may be accepted.

Calculations for Tier 1

Table 39: Tier 1 calculations for contact exposure (Vegetation margin)

Parameters	Nomenclature	Value	Unit	Origin	Source
Input					
Application rate	AR		g/ha	S	
Exposure factor for dietary exposure Vegetation margin	EF_{co}	0.381	[-]	D/S	TAB ENV 248 (2022)
Body surface factor	BSF	0.0114 (HB) 0.0146 (BB) 0.00184 (SB)	dm^2/bee	P	Table 9
Output					
Predicted Exposure Quantity for contact exposure	PEQ_{co}		µg/bee	O	
Calculation					
$PEQ_{co} = AR \times EF_{co} \times BSF$			**Equation 7**		

5.7.3.3. Tier 2

In Tier 2, PEQ can be refined by changing the parameters of the calculations in Tier 1 presented above.

Specific input parameters for Tier 2

AR — Reduced application rate may be considered provided that it is confirmed by efficacy data. Ref. Table 39.

EF_{co} — For Vegetation margin scenario – applicants may provide experimental data on the basis of which the default drift value described above may be refined. See Table 39.

6. Effect assessments in lower tiers

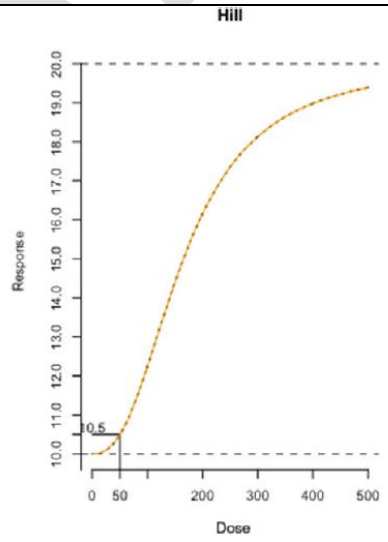
The effect assessment of biocides generally relies on point estimates (EC_x, LC_x, NOEC etc.). However, the effect assessment for bees described here is based on the concept of dose-response relationships described by mathematical models.

The goal of the effect assessment is to identify the relevant toxicity endpoints for the exposure in question. In order to do this, toxicity endpoints from four risk cases (i.e., acute oral, acute contact, chronic, larvae; indicated by the suffix *j*) are needed. These are used together with the predicted exposure quantity PEQ_j, to estimate the levels of risk of the biocide use in question.

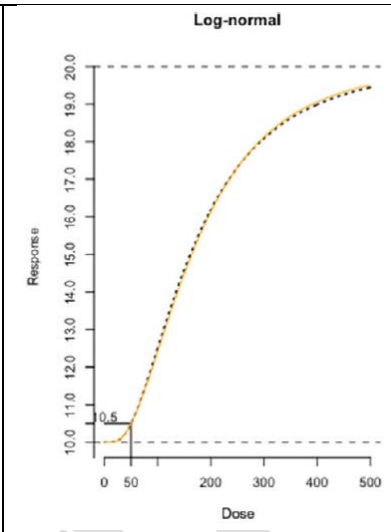
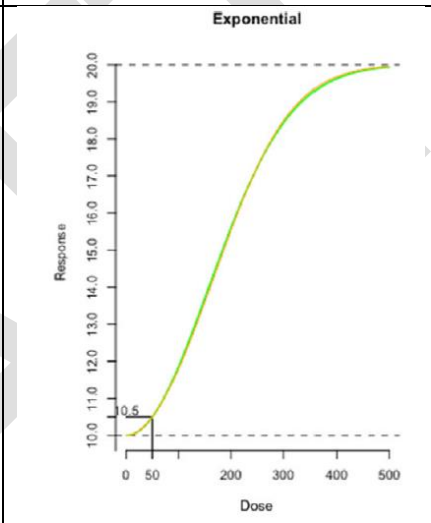
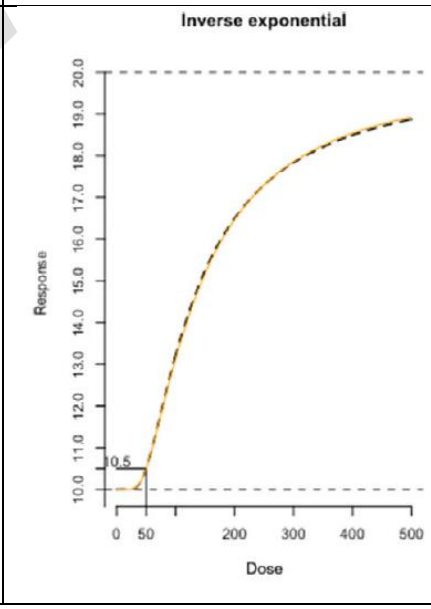
The effect parameters (*LD50*/*Inflection Point* and *slope*) are derived by fitting a dose-response curve (hereafter DRC_j) to the raw data of each standard laboratory test by using the four models below. The model which gives the best fit in describing the dose-response relationship for each risk case is then chosen and its parameters used in the effect assessment. The effect parameters resulting from the four dose-response models can be calculated with the R4EU-Calculator of the EFSA bee tool[11] and the effect parameters resulting from the model with the best fit are chosen for further effect assessment.
The74ffectt endpoints are the combination of the chosen dose-response model and the values of its parameters for each specific DRC_j.

One of the four models presented in Table 40 is used to derive a DRC_j for each risk case for the bee effect assessment.

Table 40: Overview of dose-response models to be used in the bee effect assessment of biocides. In all equations it is assumed that the effect axes of the DRC ranges from 0 to 100 and is expressed in % effect. The upper limit of the DRC is always fixed to 100, i.e., 100% mortality.

Dose response model	Illustration of models
Log-logistic (Hill) $$PIE_j = 100 \cdot \frac{1}{1 + \left(\frac{LD_{50,j}}{PEQ_j}\right)^{slope_j}}$$	

[11] Note: The EFSA Calculator tool is currently under development and will be made publicly available

Log-Normal $$PIE_j = 100 \cdot \Phi\left(\log\left[\left(\frac{LD_{50,j}}{PEQ_j}\right)^{slope_j}\right]\right)$$	
Exponential (Weibull-1) $$PIE_j = 100 \cdot \exp\left(-\left(\frac{IP_j}{PEQ_j}\right)^{slope_j}\right)$$	
Inverse Exponential (Weibull-2) $$PIE_j = \exp\left(-\left(\frac{IP_j}{PEQ_j}\right)^{slope_j}\right)$$	

Curve parameters: $LD_{50,j}$: Lethal concentration resulting in 50% mortality; IP_j: Inflection Point, where the convex function of the curve changes to a concave function of the curve. PEQ_j: predicted exposure concentration; PIE_j: predicted individual effect (see also chapter 7.1.1.); $slope_j$: slope parameter, steepness of DRC; Φ : Probability distribution function phi, or standard normal distribution function.

A detailed overview of the suite of dose-response models to be used in the bee effect assessment can be found in Chapter 6 of the EFSA Supplementary Document (EFSA, 2023). Background information can be found in the BMD Guidance Document (EFSA Scientific Committee, 2022).

To ensure the use of appropriate effect parameters in risk assessment, the following aspects should carefully be considered.

- Definition of hazard parameters in experimental studies indicated by the legal requirements (Section 6.1)
- Dealing with equivalent studies performed with the same test item and the same species (Section 6.2)
- Derivation of a surrogate dose-response beyond the tested range (Section 6.3)
- Consideration of time-reinforced toxicity (Section 6.4)
- Extrapolation of the hazard parameters between species (Section 6.5).

6.1. Definition of hazard parameters in experimental studies indicated by the legal requirements

6.1.1. Legal requirements

Under Regulation (EC) No. 528/2012, the information requirements for active substances and biocidal products are set in Title 1 of Annex II and III of the BPR, respectively. For active substances with regard to bees they are described in point 9.5.1 (honey bees) and in point 9.5.2 (applicable for bumble bees, solitary bees and other non-target terrestrial arthropods) of Annex II. For biocidal products they are described in Annex III under point 9.3.

A dossier should contain toxicity tests that are necessary to identify the potential toxic effect related to a certain exposure pathway for a biocide (described in Chapter 4). The tests should be performed according to the standard guidelines, such as OECD test guidelines, or existing protocols (pending validation and adoption as new test guidelines, see overview in Table 41). In addition, relevant information from public literature and non-guideline studies can be used. Generally, the relevance and reliability of all available studies should be considered for the overall selection of endpoints.

6.1.2. Toxicity studies

Concerning the available standard test guidelines as well as the evaluation of submitted bee tests please refer to EFSA Bee guidance Section 6.1.2.

For the assessment of biocides, toxicity studies for bees should be provided if

1. the active substance(s), has an insecticidal mode of action

   and

2. there is a relevant exposure of the biocidal product to bees

An insecticidal mode of action is usually assumed for active substances (to be) approved in PT18.

Concerning the relevant exposure of bees, please refer to ECHA Bee guidance chapter 5.

Generally, data according to test guidelines presented in Table 41 should be provided for the effect assessment of bees.

For the effect assessment of bees, data should generally be submitted for all bee types for which internationally validated and standardised test guidelines are available. Data on honey bees is considered a mandatory requirement. Data on other bees may in addition be requested if it is

relevant for the assessment.

Table 41: Overview of the currently available standard test guidelines for the effect assessment of honey bees, bumble bees, and solitary bees.

Honey bees	Acute oral toxicity	OECD 213
	Acute contact toxicity	OECD 214
	Chronic oral toxicity	OECD 245
	Toxicity to larvae	OECD GD 239
Bumble bees	Acute oral toxicity	OECD 247
	Acute contact toxicity	OECD 246
Solitary bees	Acute oral toxicity	according to Roessink et al. 2019
	Acute contact toxicity	according to Roessink et al. 2017

If the applicant provides only the above-described data for honey bees, this will also be accepted for the risk assessment for bees.

In this case, toxicity extrapolation factors (T_{ef}) have to be applied (please refer to section 6.5) to consider differences in size within and between the 3 bee groups (HB, BB and SB).

6.1.3. Active Substances and Biocidal Products

When the toxicity of the biocidal product cannot be reliably predicted from the active substance, studies performed with the biocidal product may be required (Annex III, BPR).

In the case of a mixture, that is a biocidal product with more than one active substance, the toxicity of the mixture cannot be predicted based on the data of the active substance alone. In this case data on the mixture are always required (see Table 42). For the risk assessment approach for biocidal products containing more than one active substance, see ECHA Bee guidance Chapter 12.

For biocidal products containing only one active substance, at least acute (contact and oral) studies are required for both the active substance and the biocidal product, as basis for a toxicity comparison between the active substance and the biocidal product. For the biocidal product a chronic toxicity study and a honey bee brood study can be waived, if based on the comparison between acute toxicity studies, the biocidal product results in a comparable or lower toxicity than the active substance. A ratio of 3 is used to identify a potential higher toxicity of the biocidal product based on the acute toxicity endpoints (EFSA Bee guidance section 6.7.1). Therefore, if:

- $LD_{50,acute}$ (a.s.) / $LD_{50,acute}$ (biocidal product) > 3: acute, chronic and brood data for both the active substance and the biocidal product must be provided.
- $LD_{50,acute}$ (a.s.) / $LD_{50,acute}$ (biocidal product) ≤3: no further data on the biocidal product are needed.

If several equivalent tests are available for the same species and the same test substance, please see Section 6.2. If for a toxicity endpoint only a right-censored, undefined LD_{50} value is available (datapoint is above a certain value, but unknown by how much: "LD_{50} > .."), please see sections

6.2 and 6.3. Further considerations about the comparison between active substance and the biocidal product are given in Section 6.7.1.

Table 42: Summary of the data requirements for the active substance and the biocidal product (on the basis of EFSA Bee guidance)

Tier 1 study type	***Study with active substance***	***Study with formulation required?***	
		Formulation with one active substance	***Formulation with more than one active substance***
Acute oral	*Yes*	*Yes*[a]	*Yes*
Acute contact	*Yes*	*Yes*[a]	*Yes*
Chronic oral toxicity to adults	*Yes*[c]	*Pending on the comparison between acute studies*[b]	*Yes*
Toxicity to larvae	*Yes*[c]	*Pending on the comparison between acute studies*[b]	*Yes*

[a] *Acute studies with the formulation can be waived when the toxicity can be predicted on the basis of the active substance (e.g. when the formulation consists of the active substance only, or of the active substance in water).*

[b] *Generally, a study with the active substance will be sufficient; however, if there is an indication from the acute oral study that the formulation is more toxic than the active substance, then the formulation should be tested. In determining whether there is a difference then the endpoints should be expressed in terms of active substance. If the acute formulation endpoint expressed as active substance is more toxic by at least a factor of 3 than the acute endpoint for the active substance, then it can be assumed that the formulation is of greater toxicity and hence chronic and larval testing should also be carried out using the formulation. If the difference is less than a factor of 3, then testing adult chronic and larval toxicity with the active substance is sufficient.*

[c] *In case of poorly soluble substance, a study on the formulated product might also be appropriate as surrogate if higher solubility levels are expected with the formulated product under the test conditions.*

6.2. Combining equivalent studies performed with the same test item and the same species

Sometimes multiple equivalent tests on the same endpoint and test substance are available e.g., several acute contact tests with honey bees and a certain test substance. In such cases averaging of the test results is proposed, before checking whether the formulation shows higher toxicity compared to the active substance (see Sections 6.1 and 6.7).

If multiple equivalent studies are available, the datasets can in principle be merged before fitting any dose-response model. If the survival in the control differs among the experiments, it may be appropriate to transform the data using a corrected survival before merging.

In cases, where the results of equivalent studies differ considerably, fitting a single model to a merged dataset will lead to a large uncertainty. In such case, it is worth exploring whether the recorded difference is due to any known external factor, or whether the experiments differ in their level of reliability. It should be decided case-by-case if such experiments are excluded from the effect assessment.

6.3. Derivation of surrogate dose-response beyond the tested range

For some substances, e.g., substances with low toxicity or 'difficult-to-test' substances with low solubility, the highest tested dose or the 'limit dose' is expressing an effect <50%. In this case, the $LD_{50,j}$ is often referred as right-censored, undefined value (e.g., $LD_{50,j}$ > 100 µg a.s./bee).

In these cases, the experimental data does not allow to derive a full dose-response curve. However, a surrogate dose-response curve can still be derived by making some conservative assumptions.

In the context of the proposed risk assessment scheme, the most important part of the dose-response is the one below the LD10. This is because an effect higher than 10% would immediately trigger a concern of high risk. Thus, the derivation of a surrogate dose-response curve is mandatory in case of limit test experiments (i.e., tests with a single treatment dose) and in case of dose-response experiments when the maximum dose did not trigger an effect >10%. In every other case, the data may be sufficient to describe at least the left part of the dose-response, and in such case the use of a surrogate is not needed.

Whenever it is not even possible to estimate a partial dose-response relationship (at least up to 10% effect), it is proposed to use the log-logistic (or Hill) dose-response model. This is mainly defined by a slope and the LD50 (corresponding to the inflection point, see EFSA Supplementary Document Section 6 for further details).

In general, for any specific dose *x*, a shallower slope will lead to a prediction of higher effects for any dose < *x* (see Figure 5).

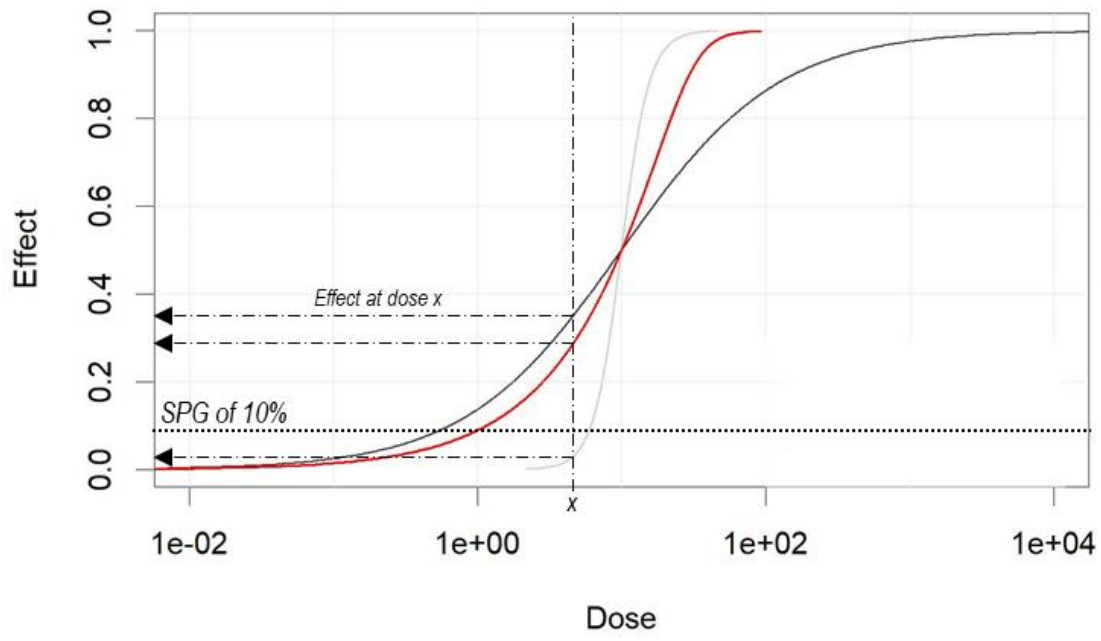


Figure 5: Illustration of the effects resulting from a dose *x* for dose-response curves with different slopes. The difference in the slope of the shown DRCs results in higher effects for shallower dose-response curves. The shown DRCs are described by the log-logistic model (Hill).

With regards to the risk assessment scheme proposed in the ECHA Bee guidance, any predicted exposure PEQ_j equivalent to a dose causing effects >10% will immediately trigger a high risk at the Tier 1 (see Chapter 7). This means that underpredicting effects above 10% has no consequences on the outcome of the risk assessment, as a risk is triggered anyway. On the other side, taking a conservative approach when predicting effects in the range 0-10% is important, as their combination will determine the outcome of the risk assessment (see Chapter 7).

As a conservative approach, a log-logistic dose response with a default $slope_j$ of 1.43 can be used whenever a specific value cannot be reliably determined from the experimental data. The

generic slope value of 1.43 corresponds to the 10th percentile of the slope distribution, based on an analysis of log-logistic dose-response curves obtained from a large number of substances. It is expected to predict an effect higher than the "true" effect in 90% of the times (see EFSA Supplementary Document Section 6.3). Using this generic slope value is considered conservative enough and is consistent with the SPG of 10%.

Once $slope_j$ is fixed, a surrogate $LD_{50,j}$ can be derived. This is done by multiplying the highest (or single) tested dose by an appropriate extrapolation factor (see Table 43). These extrapolation factors can be applied to all kind of tests, as no significant differences among slopes were recorded between groups of substances and test types.

Table 43: Extrapolation factors to derive a surrogate $LD_{50,j}$. (from EFSA Bee guidance)

*Effect observed at the highest tested dose**	*<10%effect*	*≥10 to< 20% effect*	*≥20 to-<30% effect*	*≥30 to <40% effect*	*≥40 to <50% effect*
Extrapolation factor applied to highest tested dose	*4.6*	*2.6*	*1.8*	*1.3*	*1*

6.4. Time-reinforced toxicity (TRT)

The TRT of an active substance is demonstrated when the toxic effects induced after a long period of exposure to low doses are higher than the toxic effects of a short period of exposure to higher doses. In addition, depending on the properties of the active substance, the impact of low doses on bees may be underestimated by laboratory tests where the exposure period is shorter than the environmentally relevant exposure time. Therefore, the time reinforced toxicity should always be assessed (see Chapter 8).

The TRT assessment is based on the data of the honey bee chronic toxicity study. However, the study should be correctly designed to be used for the TRT assessment. If the available standard chronic study is not sufficiently reliable or if the TRT of a substance is identified, a further chronic toxicity study might be required to refine the data.

The TRT assessment allows the determination of the toxicity parameters (i.e., $LDD_{50,\,TRT}$ and the $slope_{TRT}$) that cover the whole honey bee lifespan. If the substance shows TRT properties, the lifespan dose-response obtained from the TRT assessment substitutes the 10-days dose-response obtained directly from the chronic testing with honey bees.

6.5. Extrapolation between species

The lack of toxicity data for bumble bees and solitary bees makes it difficult to assess the risk of biocides for these bee groups. In order to derive suitable extrapolation factors, the question of how LD_{50}s differ among bee species has been investigated from different perspectives (details about the analysis are available in EFSA Bee guidance Section 6.5 and EFSA Supplementary Document).

In some ecotoxicity experiments weight measurements *for different bee species allowed establishing a generic (substance-independent) relationship between LD_{50} and bee weights, for a representative number of European bee species (~10%). This data was used to derive toxicity extrapolation factors (Tef, reported in Table 44) from standard species (A. Mellifera, B. terrestris, O. cornuta and O. rufa) to smaller bumble bees and solitary bees, in order to protect at least 95% of European species with 95% confidence. This is considered a very conservative approach, that can be revised if more information will become available*.

The Tef values take into account that smaller bees are characterised by smaller $LD_{50,j}$, resulting in a higher effect. With regards to exposure estimates, however, smaller bees are characterised

by lower exposure, which also depends on body weight and body surface (see EFSA Bee guidance Section 5.3.5 and 5.2.3, respectively).

For larvae, defining a Tef value was not possible as no suitable information is available to relate neither the LD_{50} nor the predicted exposure levels to the bee size. Exposure estimates for larvae are based on Bombus terrestris for bumble bees and Osmia species (O. rufa and O. cornuta) for solitary bees (see Chapter 5). As the larvae of these species are not significantly smaller than honey bees, a Tef = 1 is proposed to extrapolate from honey bee larvae to bumble bees and solitary bee species (Table 44).

Table 44: *Toxicity extrapolation factors (Tef). Standard $LD_{50,j}$ should be divided by these factors to obtain an estimate of an $LD_{50,j}$ protective of 95% of the species in the group* (for details see chapter 6.5 of EFSA Bee guidance and Supplementary document, respectively).

Category - extrapolation from -	***Tef for extrapolation to***	
	5th percentile BB weight	*5th percentile SB weight*
Standard honey bee adult (A. mellifera worker)	*2.4*	*171*
Standard bumble bee adult (B. terrestris[a] worker)	*6.6*	*-*
Standard solitary bee adult (O. rufa ♀) (O. cornuta ♀)	*- -*	*144 307*
Standard honey bee larva (A. mellifera worker)	*1.0 [b]*	*1.0 [b]*

[a] OECD test guidelines No. 246 and 247 were also ring tested with B. impatiens. If data are available with this species, both Tef and food consumption values should be recalculated based on the appropriate body weight. For Tef, the formula is available in the Supplementary document under Section 6.5.4. Camp et al. (2020) reported an average weight of 178 mg for B. impatiens.
[b] Tef not meant to address the 5th percentile species in terms of weight, but rather Bombus terrestris for bumble bees and Osmia species for solitary bees, i.e., species used to estimate the exposure levels to bumble bees and solitary bees.

The appropriate extrapolated $LD_{50,j}$ for bumble bees and solitary bees is calculated as follows:

$$Extrapolated\ LD50,j\ = \frac{(surrogate)Standard\ LD50,j}{Tef}$$

If data are available on other standard species, those should be used in the derivation of the extrapolated $LD_{50,j}$ for their specific bee group.
The Tefs presented in this section are estimates based on the relationship between LD_{50} and bee weight. Weight alone, however, is not the only driver of the LD_{50}.

Investigating the generic 'intrinsic' sensitivity of various species, *A. mellifera was found to be among the most intrinsically sensitive species (see Section 6.5.3.7 of the EFSA Supplementary document), indicating that the derived Tefs are likely protective, despite some uncertainty remains*.

With regard to the shape (i.e., the slope) of dose-responses for bees other than honey bees almost no information is available in the literature. Nevertheless, there is no particular indication that the shape of the dose-response, which is mainly driven by toxicokinetic and toxicodynamic aspects, should vary significantly. Therefore, the DRC_j obtained from tests carried out with honey bees can also be used for assessing the same endpoint for other bee groups. In case *dose-response* is available from tests with (standard) bumble bees and/or solitary bees, these values should be used to derive the representative DRC_j (for details see EFSA Bee guidance Section 6.5).

6.6. Implications of time-course of the effects on exposure considerations

Expressing the exposure in terms of time-weighted average for substances with fast kinetics, where the initial exposure is the one causing most of the effects (even in conditions of constant exposure), may significantly underestimate the effects under the time-variable exposure expected in the field. Therefore, if the observed effects are not due to the exposure duration (Figure 6A) *but largely due to the initial exposure* (Figure 6B), *the averaging time window w* (see EFSA Bee guidance section 5.3.13) *is reduced to 1 day*.

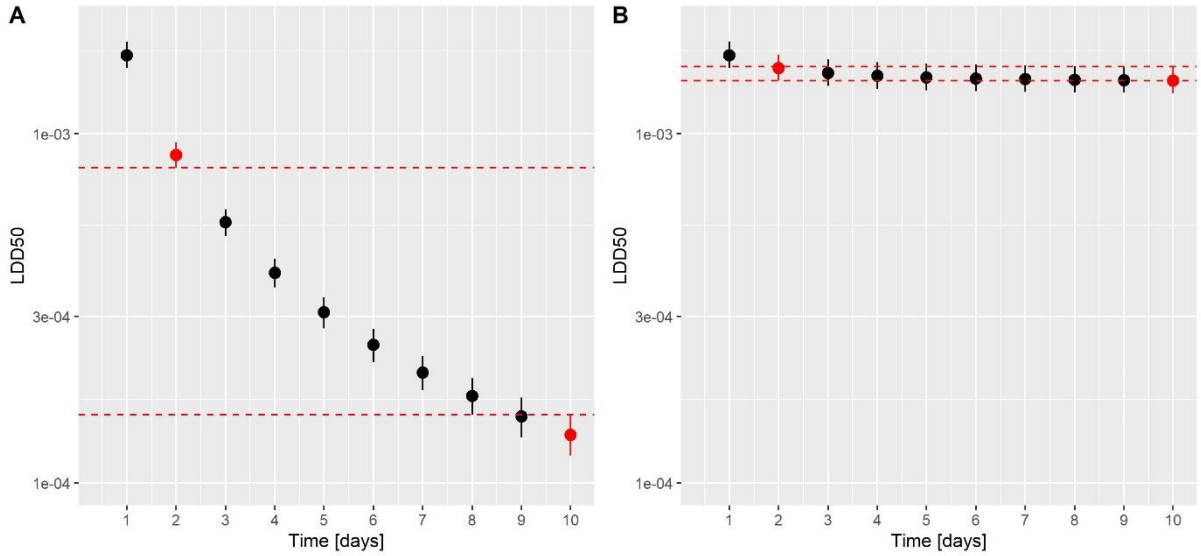


Figure 6: ***Examples of different situations concerning the time-course of effects. In panel A, full expression of effects is depending on the exposure time. Uncertainty ranges of the LDD50 at 2 and 10 days are well separated (red dotted lines show lower limit of LDD50 at 2 days and upper limit at 10 days). In panel B, effects are almost entirely expressed after a short time. Uncertainty ranges of the LDD50 at day 1 and day 10 overlap. In such cases, the use of a time-weighted average for estimating the exposure does not seem appropriate*. (EFSA Bee guidance)**

In such a case, there is practically no difference between acute and chronic exposure and combining the effects of chronic and acute dietary exposure means counting twice the same process (e.g., acute exposure). As a consequence, the acute dietary case is excluded from the overall estimation of the risk (Section 7.1.3), while the chronic risk case is determined by the 10-days chronic DRC and a modified exposure level which accounts for a reduced time window w = 1 day.

Such rapid expression of effects is opposite to the phenomenon of time-reinforced toxicity (TRT). Therefore, if TRT has been observed (including substances for which no effects were seen in the chronic test), no further check is needed. On the contrary, if TRT is not observed, the temporal trend of the LDD50 has to be checked so see whether effects are expressed immediately after the initial exposure. This can be easily done after fitting a chronic test dataset to GUTS models (something that would anyway need to be done for ruling out TRT properties – see Chapter 8). If the LDD50 after 2 days and after 10 days are significantly different or present a ratio > 3, it can be concluded that the exposure time plays an important role in the overall expression of effect, and thus no modification of the standard time window w is needed.

6.7. Summary of the selection of hazard parameters for the risk assessment

To select the appropriate DRC to be used for risk assessment for each group of bees and each risk case *j*, it is necessary to consider all the elements discussed above.

Standard test protocols for honey bees covering the different risk cases, are generally the starting point to derive the effect parameters for other bee species as well, considering the inter-species sensitivity. Sometimes other tests with bumble bees (*Bombus terrestris*) and solitary bees (*Osmia spp*.) are also available and should be used as a reference for the group of bees they belong to.

6.7.1. Effect parameters for the risk assessment of honey bees

In order to select the representative DRC_j for any test substance (either an active substance or a biocidal product containing one active substance) it is necessary to consider all the available data. The procedure is summarised in steps 1 to 4 described below:

Step 1 – if more than one equivalent test available (Figure 7)

If several equivalent honey bee tests with the same test substance are available, and their outcome is not considerably different, the datasets should be merged before fitting any dose-response model (Section 6.2).

If the outcome of different experiments is considerably different, and the difference is due to any known external factor, or due to differences in reliability, datasets should be selected or excluded on a case-by-case decision.

If none of the available tests allow the derivation of at least a partial dose-response curve covering at least effects ≥ 10% (e.g., in the case of limit tests), a surrogate dose-response can still be derived by applying the appropriate extrapolation factor to the maximum (or unique) tested dose to derive a surrogate LD50. A log-logistic model with a worst-case default slope of 1.43 should be used in these cases as a surrogate DRC_j (Section 6.3).

STEP 1 (section 6.2-6.3): repeat for all risk cases j and for every test item (a.s. and biocidal product).

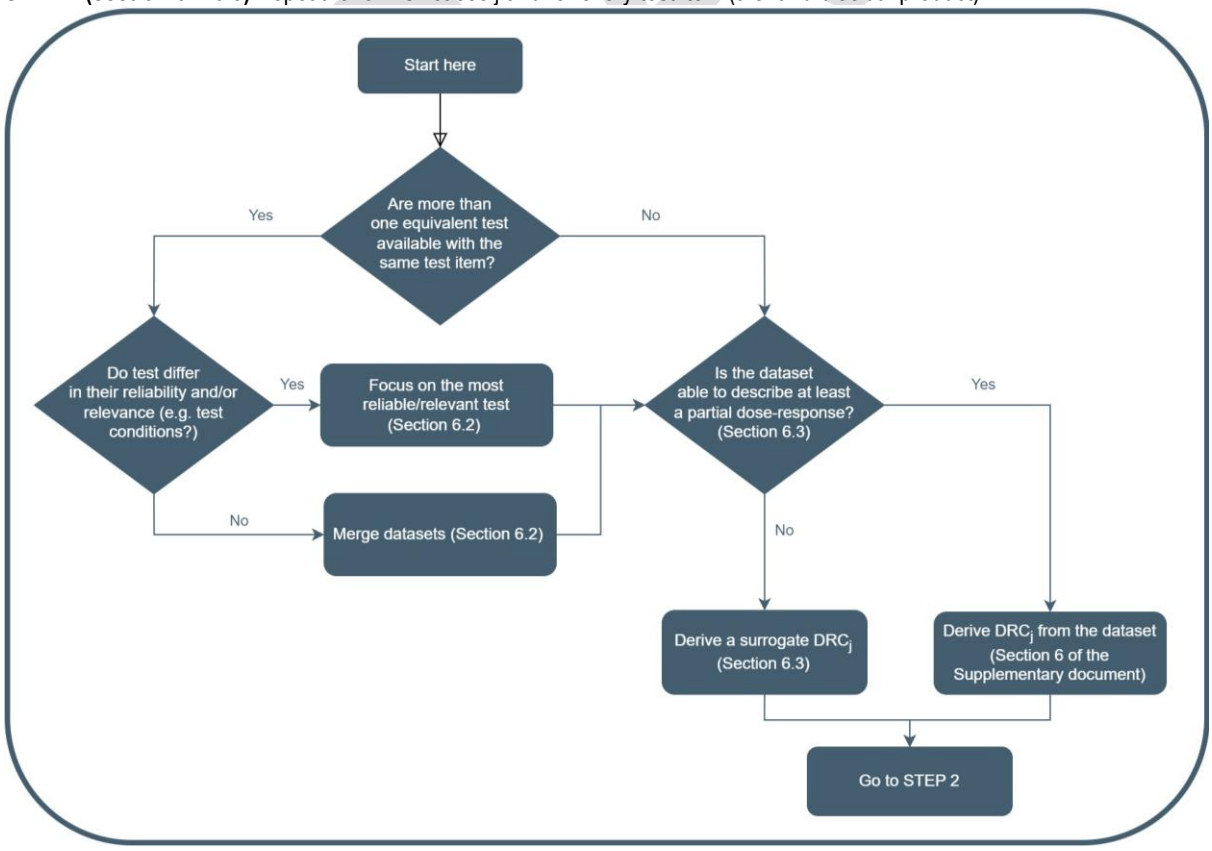


Figure 7: ***Flowchart illustrating the Step 1 of the process underpinning the selection of the hazard parameters***

***for the risk assessment of honey bees. In this picture, tests are considered equivalent when they relate to the same risk case*. Repeat for all risk cases j and for every test substance (a.s. and biocidal product)**

Step 2 – selecting most appropriate DRC_j (a.s. or biocidal product) (Figure 8)

In order to decide whether the DRC_j of the active substances or the biocidal product is more appropriate for honey bee risk assessment, the difference in the LD50 of a.s. and product (both expressed in terms of active substance) has to be explored. If the $LD50_j$ for the product is more than a factor of 3 below that of the active substance, the effect parameters of the product must be selected for the risk assessment of the active substance in the context of its inclusion/renewal (Section 6.1**Error! Reference source not found.**).

If only one of the DRC_j of the active substance and the product is not a surrogate, this DRC_j should be used for the risk assessment, unless in one of the studies thereby higher effects at comparable doses are neglected[12]. When both DRC_j (active substance and product) are surrogates, additional case-by-case considerations must be made with regard to observed mortality and tested doses. For example, if the top/limit dose caused no mortality for either the biocidal product or the active substance, a comparison is not meaningful. In such case, it would be appropriate to use for the risk assessment the surrogate dose-response obtained from the highest tested dose (expressed as active substance).
This comparison is normally performed on acute data; however, if the product is acutely more toxic, then also chronic and larvae data should be provided for the product and included in the comparison. If the product is more toxic, the risk assessment for the active substance should be based on the effect parameters derived from tests with the product.

In case of poorly soluble substances, where higher solubility levels are expected with the product under the test conditions, chronic and larval studies should be carried out uniquely with the formulated product. In this case, the DRC_j derived with the product should be considered (as surrogate) for the active substance.

STEP 2 (section 6.1): repeat at least for the two acute risk cases. Apply to chronic dietary and larval risk cases as well in case tests with the biocidal product are available.

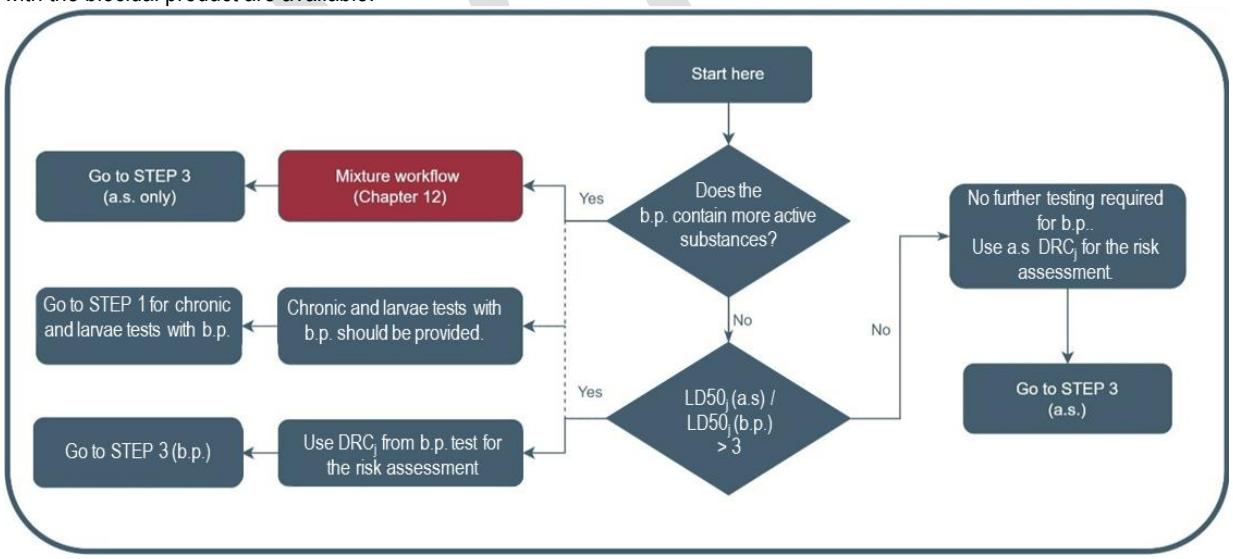


Figure 8: ***Flowchart illustrating the Step 2 of the process underpinning the selection of the hazard parameters***

[12] Example: a study with the active is carried out as limit test at a dose x triggering 30% effect (only surrogate dose-response possible). The study with the formulated product is instead carried out as a proper dose-response. The effect in this second study at a dose ≈ x is considerably lower than 30%. In this case the surrogate dose-response obtained with the active should be retained for the risk assessment.

for the risk assessment of honey bees. Note that the comparison of the $LD50_j$ between active substance and PPP entails additional consideration in case of surrogate DRC_j (see text).

Step 2 is not necessary if the biocidal product contains additional active substances. In such case, the mixture workflow should be followed (see Chapter 12).

Step 3 – Selection of effect parameter for chronic RA of honey bees (Figure 9)

When selecting the effect parameters for chronic risk assessment of honey bees, it should be considered whether the active substance (and the product, if this is triggered at the Step 2) shows time-reinforced toxicity (see Section 6.4 and Chapter 8, and for further details chapter 8 and Annex G of the Supplementary document of the EFSA Bee guidance). If this is the case, the 10-days chronic dose-response should be substituted by the life-span dose-response and an additional risk assessment for winter scenario is triggered, using a life-span dose-response for long-lived winter bees.

STEP 3 (section 6.4, chapter 8 and Annex G of EFSA Supp. document): only for chronic

- a.s.: all cases
- biocidal product only if triggered at STEP 2 and only in case it contains a single a.s.

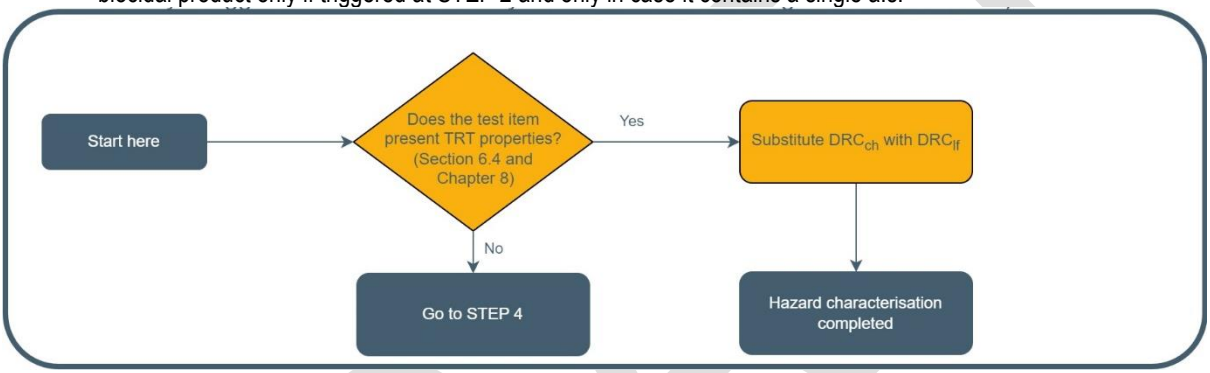


Figure 9: ***Flowchart illustrating the Step 3 of the process underpinning the selection of the hazard parameters for the risk assessment of honey bees.***

Step 4 – If effects are driven by initial exposure (Figure 10)

When effects are expressed immediately and mainly driven by the initial exposure (even in conditions of constant chronic exposure), estimating the exposure in terms of time-weighted average may significantly underestimate the effects in the field (see Section 6.6). In this case the averaging time window *w* (see EFSA Bee guidance Section 5.3.13) is reduced to 1 day. In this case the dietary acute and chronic risk cases are conceptually overlapping, and the first one is thus excluded from the combination of effects in the risk assessment (Section 7.1.3).

This situation will not occur if a substance presents TRT properties and/or if no effects are seen in the chronic test. Section 6.6 describes how to check whether effects are expressed immediately after the initial exposure.

STEP 4 (section 6.6): only for chronic (a.s. and biocidal product if evaluated at STEP 3)

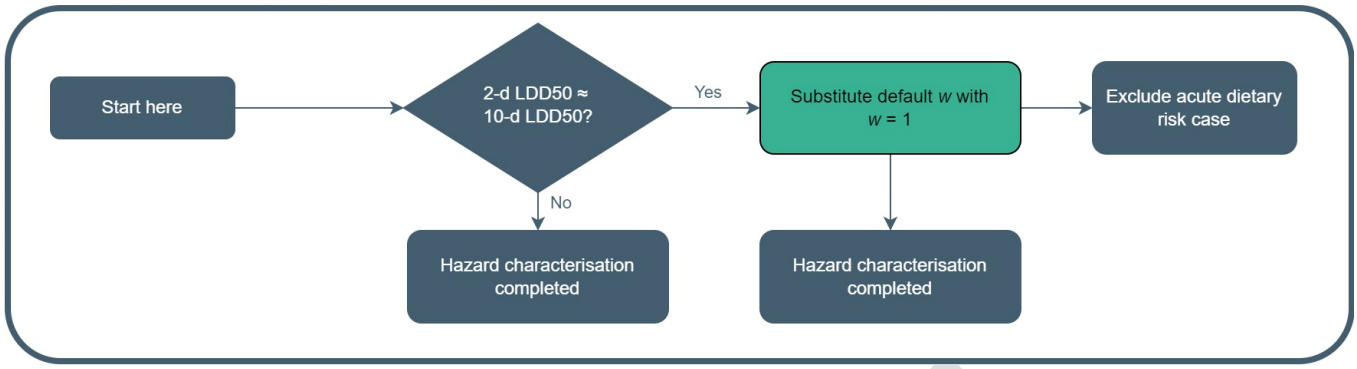


Figure 10: ***Flowchart illustrating the Step 4 of the process underpinning the selection of the hazard parameters for the risk assessment of honey bees. The effect of the exposure length is considered minor if the LDD50 after 2 days and after 10 days are not significantly different and present a ratio < 3.***

6.7.2. Effect parameters for the risk assessment of bumble bees

*For bumble bees, OECD TG 246 and 247 acute tests may be provided in the dossier with both active substance and representative biocidal product. In addition, relevant literature data may be available. The treatment of the effect parameters (i.e. DRC_j) from any available test with the standard species (Bombus terrestris and, less frequently, Bombus impatiens) should follow steps 1-2 summarised in Section 6.7.1 (*Figure 7 *and* Figure 8*). In contrast Steps 3-4 most likely are not applicable as they are honey bee specific, except if a standard chronic test with bumble bees is available.*

As the bumble bee group includes many untested species (see Section 1), toxicity extrapolation factors (Tef) should be used to cover the inter-species differences and to obtain the relevant extrapolated DRC_j (Section 6.5).

In all cases where no bumble bee data are available (e.g. chronic and larval effects) the extrapolated DRC_j should be determined by applying the appropriate Tef to the DRC_j chosen for honey bees.

6.7.3. Effect parameters for the risk assessment of solitary bees

If studies based on publicly available test protocols or draft OECD TG are available (likely for acute exposure only), they can be used to derive the effect parameters for the solitary bee risk assessment. When this is the case, the DRC_j from those studies could be used to obtain the extrapolated DRC_j after applying the appropriate Tef.

If no such test are available, the honey bee effect parameters should be used by applying the appropriate Tef to the honey bee DRC_j and using the respective DRC_j as explained in Section 6.5.

6.8. Options for refinement

In the rare cases, where for an active substance or biocidal product additional studies are available, there are two possibilities for a refinement of the Tier-1 effect assessment. In this case the EFSA GD considers two approaches (for further details see EFSA Bee Guidance Section 6.7):

- *The geometric mean approach;*
- *The species sensitive distribution (SSD) approach.*

Because of the lack of standardised test guidelines for many species and the general lack of knowledge on inter-species variability in the dose-response, it is currently not recommended to use the geomean or the SSD approach for bees. Nevertheless, for the time being, effect

information for multiple species could be considered in a weight of evidence, acknowledging that increasing the current level of knowledge would certainly improve the accuracy of the risk assessment in future.

7. Lower tier risk assessment

The aim of the lower tier risk assessment is to apply the agreed specific protection goal (SPG) of maximum 10% colony size reduction for honey bees to the proposed methodology, resulting in a conservative assessment which simultaneously identifies active substances of unacceptable risk whilst excluding the substances of low risk from further evaluation.

The suggested approach for the lower tier risk assessment does not focus on single endpoints but combines the effects of different endpoints (which are extrapolated from the individual to the colony level), using the concept of response addition (Biss, 1939). This calculation method takes into account that in real life, biocidal products can affect a honey bee colony via different endpoints and routes of exposure (see Section 1.4). Therefore, instead of determining a predicted no effect concentration (PNEC) by applying suitable assessment factors to cover for the degree of uncertainty in extrapolation from test data on a limited number of species to the real environment (BPR, Annex VI, paragraph 40. And 41), *this approach combines the predicted effects in a more mechanistic concept* (for details see EFSA Bee guidance Chapter 7).

The method presented in this ECHA Bee guidance is in line with the specific protection goal (SPG) defined by EFSA for honey bees (EFSA Bee guidance Chapter 3), which focuses on the colony/population (see Chapter 3).

This approach allows a direct comparison between the predicted effects following the exposure to a biocidal product at colony level and the SPG defined by the trigger value of maximal 10% of colony size reduction for honey bees.

The proposed procedure for such a 'combined risk assessment' consists of three successive steps:

1. *Quantification of the effects at the individual level for each risk case (acute oral, acute contact, chronic, larvae) based on standard laboratory ecotoxicological studies, and exposure estimates;*
2. *Extrapolation of the individual level effects to colony/population level effects for each risk case;*
3. *Combination of effects for all risk cases into a single predicted effect at the colony/population level*.

These steps are described in the following section. *The proposed methodology may be applied in the risk assessment of all bee groups, including honey bees, bumble bees and solitary bees*. However, it is noted that for bumble bees and solitary bees a threshold of acceptable effect has not yet been defined for the magnitude dimension of the SPG and therefore a conclusive risk assessment is not possible as also outlined in the EFSA Bee guidance.

7.1. Step-by-step explanation of the lower tier approach for honey bees

7.1.1. Step 1: Quantification of effects at individual levels

In the first step, a dose-response curve (DRC_j) is defined for each relevant bee group and life stage (for details see EFSA Bee guidance Chapter 6 and EFSA Supplementary document).

The DRC_j is then used together with the relevant predicted exposure quantity (PEQj) to calculate the predicted individual effect level (*PIE_j*). By this, the relationship between exposure to a biocide and the mortality is described and is used to indicate the proportion of bees that would be expected to die after being exposed to a specific dose.

The PIE (in unit of percentage), following the application and exposure of a biocidal product, is calculated as follows,

$$PIE_j = 100 \cdot f(DRC_j, PEQ_j) \qquad \textbf{Equation 30}$$

whereas j refers to a risk case as assessed in an experimental test such as acute-contact, acute-dietary, chronic-dietary or repeated-dose-larvae. The PEQ_j is a realistic worst-case exposure estimate for the respective exposure assessment Tier (see Section 3, Table 4), which is defined as ecotoxicologically relevant exposure quantity EREQ (uptake of a biocide by individual bee per time unit, mass of a.s.·individual-1). EREQ is represented by the dose (*PEQ_j*) in the environment as well as the dose in the ecotoxicological experiment (see Figure 11).

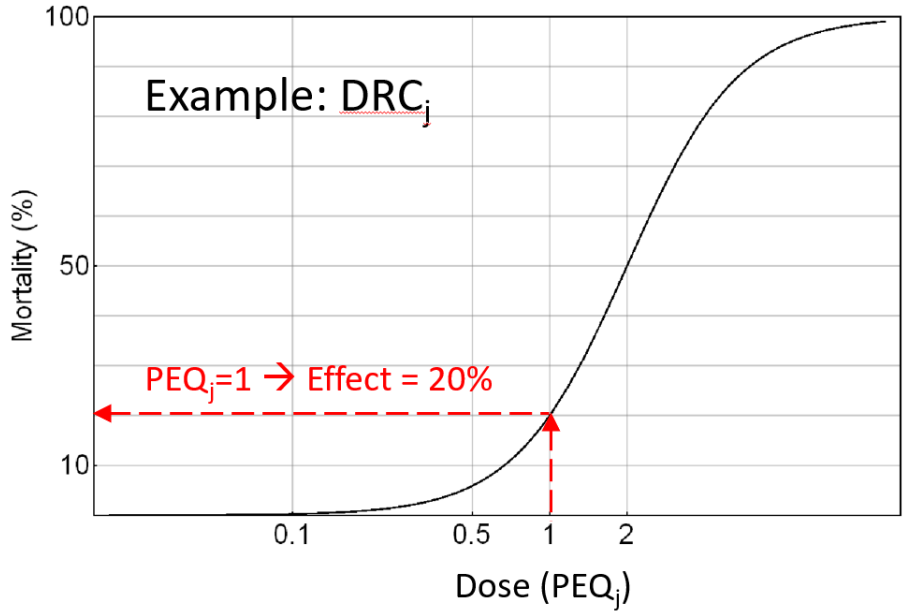


Figure 11: ***Graphical illustration of the proposed calculation for the effect on a specific endpoint using a non-linear dose-response curve DRCj. The resulting mortality (%) can be interpreted as probability of one individual to die due to exposure to a certain dose, which can also be interpreted as a percentage of a cohort of individual bees to die after exposure to the identical dose*. (EFSA Bee guidance)**

Table 45 gives an overview of ecotoxicological exposures as defined by a proper problem formulation and the derivation of effect parameters for honey bees (described in chapter 4 and 6, respectively).

Table 45: Overview of exposure and the dose-response for the different life stages of honey bees, adapted for biocides (EFSA Bee guidance)

Life stage	***Category***	***Exposure***			***Dose-response***	
		Route	***Duration***	***Quantification and time scale***	***Potency***	***Slope***
Adult	*Forager*	*Contact*	*Acute*	*From contact exp. model; biocide mass sticking on the forager after a single application*	*Determined experimentally from the acute contact test. If it cannot be derived, a worst-case surrogate is used (see Chapter 6).*	
Adult	*Forager*	*Dietary (oral)*	*Acute*	*Worst-case between the two bee roles from dietary model; biocide mass uptake per bee per day*	*Determined experimentally from the acute oral test. If it cannot be derived, a worst-case surrogate is used (see Chapter 6).*	
Adult	*In-hive (nurse)*					
Adult	*Forager*	*Dietary*	*Chronic*	*Worst-case between the two*	*Standard chronic assessment*	

Adult	*In-hive (nurse)*	*(oral)*		*bee roles from dietary model; average daily biocide mass uptake per bee during: 10 days (standard chronic assessment) - 27 days, i.e. the average lifespan of honey bee workers (for substance with TRT properties).*	*Determined experimentally from the chronic oral test. If it cannot be derived, a worst-case surrogate is used (see Chapter 6).* *TRT assessment[a]* *Determined via extrapolation from the chronic oral test.*
Larvae	*General worker*	*Dietary (oral)*	*Chronic (prolonged)*	*From dietary model; average daily biocide mass uptake per larvae during 5 days.*	*Determined experimentally from the larvae prolonged test/repeated exposure. If the slope is not available, a worst-case value is used (see Chapter 6).*

[a] When a substance has TRT properties, the risk should be evaluated for the entire honey bee lifespan for both the active (27 days) and the winter (182 days) period. Nevertheless, the winter scenario is a stand-alone assessment, which does not follow all of the steps illustrated in this Chapter. See Section 8.2.2 for more details.

7.1.2. Step 2: Extrapolation of the individual level effects to colony

In lower effect-tier assessment, toxicity endpoints are investigated in laboratory studies and effects are expressed at levels of individuals. The SPG, however, defines the relevant ecological entities as the colony (honey- and bumble bees) and the population (solitary bees), respectively. To make the lower tier risk assessment compliant with the SPG, effects need to be extrapolated from individual levels to higher levels of biological organization (i.e., colony or population). The extrapolation is based on a worst-case exposure related to a certain risk case (larvae, foragers, in-hive bees). Details on the extrapolation from individual to colony/population levels can be found in the EFSA Bee guidance section 7.1.2 and in the EFSA Supplementary document.

In the simulations conducted to support the extrapolation from individual to colony/population level it was assumed that all foragers experience an increased mortality (even without any exposure), if they leave the hive at least for one foraging flight on a day with simulated additional mortality (EFSA et al., 2021). It was further assumed that contact exposure is only relevant for foragers.

*The simulations showed that for most ecological conditions, increased forager mortality resulted in a lower effect at the colony level (i.e., in terms of strength), but for some months of the year, the simulation results were close to the 1:1 line (*Figure 12*). Therefore, for foragers a 1:1 extrapolation from individual to colony level is appropriate.*

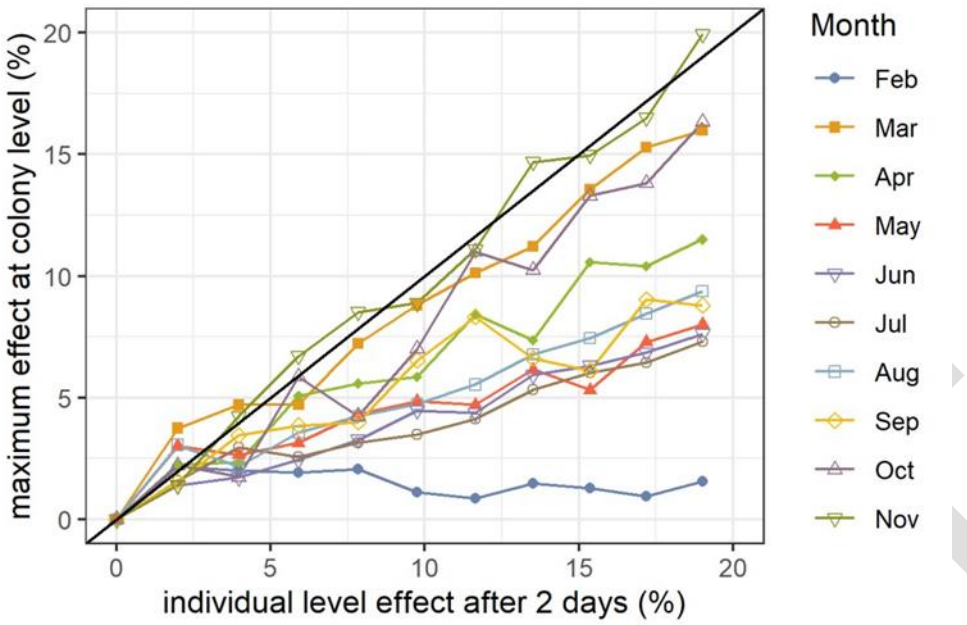


Figure 12: ***example BEEHAVE propagation: Example for the extrapolation of individual level mortality related to an assumed contact exposure to the colony level for the scenario D3 (EFSA et al., 2021). On the y-axis is the maximum observed effect at colony level after 2 days for an assumed contact exposure event in the middle of the respective 10 months in a year, indicated by single lines and symbols. The black solid line depicts the 1:1 line. It can be seen that for some months of the year, e.g., November, March and June, the effect at individual levels was more or less fully propagated to the colony level*. (EFSA Bee guidance)**

For dietary exposure (acute, chronic, or larvae), which is assumed to affect every bee in a colony via nectar and/or pollen, also a 1:1 extrapolation from individual to colony level is appropriate. Consequently, in the lower Tier risk assessment, the extrapolation step from the individual (PIE_j) to the colony level effect (PCE_j) assumes a conservative 1:1 propagation for all risk cases:

$$PCE_j = PIE_j$$ **Equation 31**

7.1.3. Step 3: Combination of effects at the colony

In the third step, effects predicted for single risk cases (PCE_j) are combined. This is justified by the consideration that under real world conditions the effects of different exposure pathways and life stages add up at the colony level, which is the ecological entity defined for the SPG for honey bees. The addition of the responses of the single risk cases is based on the mathematical model of independent action (IA, or response addition), which is used for predicting the joint effect of mixtures (Bliss, 1939). It is used to calculate an overall predicted effect at colony level (PE_{SPG}), in units of % of colony size reduction and is mathematically expressed by:

$$PE_{SPG} = 100 \cdot (1 - \prod_{j=1}^{n}(1 - \frac{PCE_j}{100}))$$ **Equation 32**

The PE_{SPG} is directly compared with the SPG of ≤10% colony size reduction for honey bees (for bumble bees and solitary bees no SPG value has been defined so far). The maximum effect is mathematically limited to 100%, independent of the number of considered endpoints. For Tier-1 the timing of the single events in the response addition is ignored, representing a conservative assumption.

7.1.4. Quantification of the contribution of a risk case to the overall predicted effect

There might be cases, where the overall predicted effect at the colony level PE_{SPG} is dominated by a single risk case. This can be assessed by quantifying the contribution of one risk case on the overall predicted effect. From the definition of the PE_{SPG}, a formula can be derived for the contribution of risk case j to the overall predicted effect:

$$\Delta_j = \frac{Ln(100 - PCE_j)}{Ln(100 - PE_{SPG})}$$ **Equation 33**

Depending on whether or not a single risk case dominates the PE_{SPG}, different options for refinement can be used in the higher tier risk assessment (see Chapter 10).

7.2. Implementation of the combined risk assessment in the tiered approach

As explained in Section 7.1, the quantification of an individual effect (Step 1 of the combined risk assessment) is driven by the PEQ_j from the exposure and by the DRC_j for the effect. Since for the effect assessment there are only very limited options for refinement (see Section 6.8), the lower tier risk assessment can be performed based on different exposure-tiers (see Chapter 3). This includes a screening-, tier1-, tier-2- and tier-3-exposure tier for the dietary risk cases and a tier-1- and tier-2-exposure tier for the contact risk case, as illustrated in Figure 13 below.

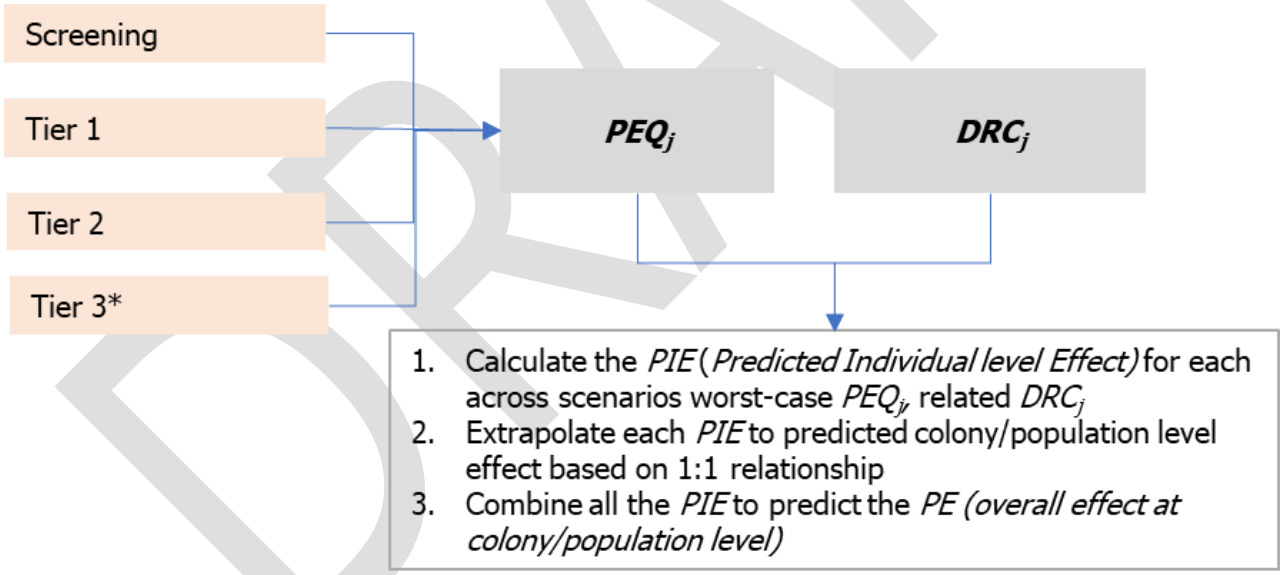


Figure 13: Combined risk assessment in relation to the exposure-tiers. (*Tier 3 applicable only for the dietary route of exposure.)

7.2.1. Screening-level risk assessment

For the screening level risk assessment, the exposure estimation for dietary and contact exposure (PEQ_j) are based on a simplified exposure model (Section 5.1.3), resulting in more conservative exposure estimations compared to the Tier 1. In this context, the PEQdi values derived from Equation 8 for each risk case for all sources of exposure for which above soil contamination is relevant need to be compared to the PEQdi values for through soil contamination presented in Table 46, where relevant. The highest of the two PEQdi values has to be considered in the risks assessment for the screening step.

The predicted individual level effect (PIE) is calculated based on the screening level PEQ_i and the related DRC_j values, for each of the risk cases (acute-contact, acute-dietary, chronic-dietary and larvae-dietary) (see Section 7.1.1). Assuming 1:1 extrapolation from individual to colony level, the predicted colony level effects (PCE) for each of the risk cases are then combined to determine the overall predicted effect at the colony level (PE_{SPG}) (see Section 7.1.3), which can be compared to the SPG. The applicant can decide whether to start the lower tier risk assessment with the screening step or directly with the Tier 1 assessment. However, if a substance presents time-reinforced toxicity (TRT), the risk assessment must start with Tier 1 exposure estimates, because exposure estimates are calculated based on different assumptions.

If the screening level risk assessment results in acceptable risk for the evaluated use, the risk assessment can stop here, and no Tier 1 assessment is consequently required. Otherwise, the risk assessment needs to proceed with Tier 1 assessment.

An example is presented below to illustrate how the calculations are to be performed to estimate the risk to honey bees at colony level by combining the different risk cases. The example calculations are performed for a hypothetical biocidal product applied to walls by spraying; thus, the source of exposure is the spraying of walls around the house (for more background, see section 5.3). The input parameters are presented in Table 46 and the calculations at the screening step in Table 47.

Table 46: Input and output parameters to derive the application rate for wall spraying around the house for Screening step/Tier 1.

Parameters	Nomenclature	Value	Unit
Input			
Application rate of product to target surface (e.g., wall, foundation)	Q_{prod}	12	g/m^2
Fraction of a.s. in the product	F_{ai}	0.001	
Treated surface (e.g., wall, foundation)	$AREA_{treated}$	125	m^2
Area of soil that is contaminated	$AREA_{soil}$	26	m^2
Output			
Application rate	AR		g/ha
Calculation			
$AR = Q_{prod} \times F_{ai} \times \frac{AREA_{treated}}{AREA_{soil}} \times 10000 = 576.9$			

Table 47: Example on how to estimate the risk to honey bees with the combined approach at screening level

Honey bees Screening level exposure					
		Dietary			**Contact**
		Acute (da)	**Chronic (dc)**	**Larvae (dl)**	**Acute (ca)**
Exposure	Factor B [-]/BSF [dm^2/bee]	9	9	9.2	0.0114
	PEQ_j [µg/bee] [a] Above soil Through soil	 PEQ_{da} = **5.19** PEQ_{da} = 0.53	 PEQ_{dc} = **5.19** PEQ_{dc} = 0.50	 PEQ_{dl} = **5.31** PEQ_{dl} = 0.542	PEQ_{ca} = 6.58

Hazard parameters (DRCj): Dose-response model (Mod) LD50/IP (e) [µg/bee][a] Slope (b)	DRCda Mod: log-logistic e = 7 b = 1.84	DRCdc Mod: log-logistic e = 9 b = 1.67	DRCdl Mod: log-logistic e = 0.7 b = 2.24	DRCca Mod: log-logistic e = 15 b = 2.23
Step 1: Predicted individual level effect (PIE)	PIE_{da} = 36.59%	PIE_{dc} = 28.52%	PIE_{dl} = 98.94%	PIE_{ca} = 13.72%
Step 2: Predicted colony level effect (PCE)	PCE_{da} = 36.59%	PCE_{dc} = 28.52%	PCE_{dl} = 98.94%	PCE_{ca} = 13.72%
Step 3: combination of effects at colony level	PE_{SPG} = 100 · (1-(1-PCE_{da}/100)· (1-PCE_{dc}/100)· (1-PCE_{dl}/100)· (1-$PCEc_a$/100)) = 100 · (1-(1-0.3659)· (1-0.2852)· (1-0.9894)· (1-0.1372)) = **99.59%**			
PE_{SPG} i.e., ≤ 10%	No (unacceptable risk identified)			

[a] Units are mentioned for brevity as µg/bee, but they are in fact µg/bee/day for chronic and µg/bee/dev. Period for larvae

In this example, PEQdi values for the above soil contamination are higher than for the through soil calculation and therefore the risk assessment is based on the former. The SPG is violated in the screening step with an overall predicted effect at the colony level PE_{SPG} = 99.59%. Therefore, the risk assessment needs to move to exposure Tier 1.

7.2.2. Tier-1 risk assessment

For the exposure Tier-1, the dietary and contact exposure estimations are performed based on the models described in Sections 5.1.1 and 5.1.2. These more complex, but more realistic model assumptions result in a lower exposure estimation compared to the screening step.

This exposure estimation is to be performed for all relevant exposure scenarios. The combined risk assessment is following the same steps as for the screening level assessment.

For the illustrative example (introduced in Section 7.2.1), Vegetation margin exposure scenario is considered applicable, nevertheless since two distinct release processes are involved – spraying and run-off/wash-off, above soil as well as through soil dietary models are relevant. Therefore, Table 48 and Table 49 present the combined approach for dietary model for above soil contamination and through soil contamination for the Vegetation margin scenario, respectively. The same application rate is assumed as presented in Table 46.

Table 48: Example on how to estimate the risk to honey bees with the combined approach at Tier 1 for dietary model for above soil contamination

Honey bees Tier-1 exposure (Dietary model for **above soil** contamination and contact)					
		Dietary			**Contact**
		Acute (da)	**Chronic (dc)**	**Larvae (dl)**	**Acute (ca)**
Exposure	PEQ_j [µg/bee][a]	PEQ_{da} = 0.415	PEQ_{dc} = 0.170	PEQ_{dl} = 0.166	PEQ_{ca} = 0.658
Hazard parameters (DRCj): Dose-response model (Mod) LD50/IP (e)[µg/bee][a] Slope (b)		DRCda Mod: log-logistic e = 7 b = 1.84	DRCdc Mod: log-logistic e = 9 b = 1.67	DRCdl Mod: log-logistic e = 0.7 b = 2.24	DRCca Mod: log-logistic e = 15 b = 2.23
Step 1: Predicted individual level effect (PIE)		PIE_{da} = 0.5503%	PIE_{dc} = 0.1323%	PIE_{dl} = 3.808%	PIE_{ca} = 0.0936%

Step 2: Predicted colony level effect (PCE)	PCE_{da} = 0.5503%	PCE_{dc} = 0.1323%	PCE_{dl} = 3.808%	PCE_{ca} = 0.0936%
Step 3: combination of effects at colony level	$PE_{SPG} = 100 \cdot (1-(1-PCE_{da}/100) \cdot (1-PCE_{dc}/100) \cdot (1-PCE_{dl}/100) \cdot (1-PCEc_a/100))$ $= 100 \cdot (1-(1-0.005503) \cdot (1-0.001323) \cdot (1-0.03808) \cdot (1-0.000936))$ $=$ **4.55%**			
PE_{SPG} i.e., ≤ 10%	Yes (acceptable risk identified)			

[a] Units are mentioned for brevity as µg/bee, but they are in fact µg/bee/day for chronic and µg/bee/dev. Period for larvae

Table 49: Example on how to estimate the risk to honey bees with the combined approach at Tier 1 for dietary model for through soil contamination

Honey bees Tier-1 exposure (Dietary model for **above soil** contamination and contact)				
	Dietary			**Contact**
	Acute (da)	**Chronic (dc)**	**Larvae (dl)**	**Acute (ca)**
Exposure PEQ_j [µg/bee]b	PEQ_{da} = 0.265	PEQ_{dc} = 0.250	PEQ_{dl} = 0.272	PEQ_{ca} = 0
Hazard parameters (DRCj): Dose-response model (Mod) LD50/IP (e)[µg/bee]a Slope (b)	DRCda Mod: log-logistic e = 7 b = 1.84	DRCdc Mod: log-logistic e = 9 b = 1.67	DRCdl Mod: log-logistic e = 0.7 b = 2.24	DRCca Mod: log-logistic e = 15 b = 2.23
Step 1: Predicted individual level effect (PIE)	PIE_{da} = 0.24%	PIE_{dc} = 0.25%	PIE_{dl} = 10.74%	PIE_{ca} = 0.0%
Step 2: Predicted colony level effect (PCE)	PCE_{da} = 0.24%	PCE_{dc} = 0.25%	PCE_{dl} = 10.74%	PCE_{ca} = 0.0%
Step 3: combination of effects at colony level	$PE_{SPG} = 100 \cdot (1-(1-PCE_{da}/100) \cdot (1-PCE_{dc}/100) \cdot (1-PCE_{dl}/100) \cdot (1-PCEc_a/100))$ $= 100 \cdot (1-(1-0.0024) \cdot (1-0.0025) \cdot (1-0.1074) \cdot (1-0))$ $=$ **11.18%**			
PE_{SPG} i.e., ≤ 10%	No (unacceptable risk identified)			

[a] Units are mentioned for brevity as µg/bee, but they are in fact µg/bee/day for chronic and µg/bee/dev. Period for larvae

For Tier-1 the more realistic model for dietary and contact exposure estimation is used, leading to a lower predicted exposure quantity. Using the Tier-1 exposure models for both contact and for dietary exposure to calculate the *PEQj* values for the Vegetation margin scenario, results in an overall predicted effect at the colony level PE_{SPG} = 4.55% and 11.18% based on dietary model for above soil contamination and for through soil contamination, respectively. Whereas non-violation of the protection goal for honey bees is identified for the former, the protection goal for the latter is violated. Therefore, in case of this example, further refinement of the *PEQj* for the dietary model for through soil contamination can be done according to Section 5.3.

With regards to the effect parameters a DRCj will not always be available for all substances and risk cases. If the derivation of a proper log-logistic dose-response curve is not possible because of limited data, in any case an LD_{50}/IP value needs to be derived, and a default (conservative) slope value can be used as described in section 6.3. Leaving out one endpoint (risk case) is not acceptable, unless an appropriate default *PIE* values is used.

If a substance is found to show time-reinforced toxicity (TRT), the predicted individual level effect for the chronic dietary risk case PIE_{dc} should be calculated differently: instead of the standard 10-day LDD_{50}, a lifespan LDD_{50}-*TRT* (covering a 27-day lifespan for the active period of honey bees) should be used, together with a PEQ_i calculated for a 27-day exposure period

(see also section 8.2.1). An additional risk assessment, covering the inactive period of honey bees during the winter period must be performed as well (see also section 8.2.2).

If the risk assessment based on the Tier-1 exposure indicates unacceptable risk (i.e. SPG not met), and it is not possible to mitigate the risk, a risk assessment based on a Tier-2 or Tier-3 is necessary. It is noted that if an appropriate Tier-2 exposure assessment is not available, and the risk was not excluded at the lower tiers, the conclusion on the risk assessment will be drawn on the basis of those lower tiers.

7.2.3. Tier-2/Tier-3 risk assessment

At the Tier-2/Tier-3 exposure assessment, several of the parameters in both the contact and dietary exposure models can be refined (see Sections 5.2 to 5.7 for details on the options for refinement and the need to generate further data). Using the refined parameter values, refined shortcut values can be calculated, which in turn can be used in the models to calculate the higher exposure tier PEQ_i.

If the risk assessment based on Tier-2 or Tier-3 exposure still indicates unacceptable risk (i.e., SPG not met), a higher tier risk assessment has to be performed (see Chapter 10).

In summary, when an unacceptable risk at colony level is not excluded, any predicted individual level effect can be reiteratively refined according to the tiered approach. Higher tier effect assessment is needed when no options are available to refine the exposure estimation.

For a biocidal use to be considered safe for bees, the overall predicted effect at the colony level (PE_{SPG}) for all relevant exposure routes as well as for all relevant exposure scenarios need to be below the defined SPG of 10% for honey bees.

7.3. Implementation of the combined risk assessment approach for bumble bees and solitary bees

Due to the lack of data, there are no defined SPGs for bumble bees and solitary bees yet, *meaning there are no values available to compare the overall predicted effect at the colony/population level PE_{SPG} to the SPG. Currently a lower tier risk assessment scheme for bumble bees and solitary bees cannot be implemented until a threshold of acceptable effects is defined for the magnitude dimension of the SPG*.

However, the guidance allows exposure estimation and effect definition for bumble bees and solitary bees, although these are characterised by considerable uncertainties due to the lack of specific data.

With no risk assessment threshold for bumble bees and solitary bees, the overall predicted effect at lower tier cannot be used to conclude on the 'acceptability of the risk'. Nevertheless, the exposure and effect data (measured or extrapolated from honey bees) for bumble bees and solitary bees can be used in the combined approach described in this chapter to highlight sensitivity differences compared to honey bees and inform on possible risks with regards to bumble bees and solitary bees.

It should be considered that there are several ecological factors that could influence the vulnerability of bumble bees and solitary bees to biocidal products differently compared with honey bees (EFSA, 2022). In general, the biology and ecology of bumble bees, and especially of solitary bees, suggest a lower resilience and higher vulnerability to stressors relative to honey bees. Although it is unclear to what extent each ecological factor contributes to their vulnerability, it is important to highlight that this is a remaining source of uncertainty in the risk assessment that is difficult to quantify due to the lack of data.

Therefore, a conservative approach to interpret the result, as also recommended by the EFSA opinion (EFSA PPR Panel, 2012), could be warranted. On the other hand, it is highlighted that specific aspects related to the biology and the interspecies sensitivity are already included in the exposure and hazard definition (see Chapters 5 and 6), although there are still uncertainties due to the lack of robust data.

In the current absence of general knowledge on bumble bees and solitary bees, it is suggested that lower tier data (standard laboratory studies) are requested for biocidal active substances (and products if necessary) to allow a better protection of these bee groups in the future.

8. Time-reinforced toxicity

According to the effect assessment approach as presented in Chapter 6, dose-response relationships (i.e., LD(D)50 values) are estimated on the basis of standard toxicity studies for which the exposure times are assumed to reflect an "acute" and "chronic" exposure time. The standard chronic laboratory toxicity test exposes bees for up to 10 days (OECD test guideline 245). Depending on the properties of the test substance (e.g., bioaccumulative properties), the toxicity based on a 10-day study could be underestimated when the toxic effects are enhanced by the exposure time.

The exposure of bees to biocides is different from that of plant protection products. Nevertheless, depending on the biocidal application, it is possible that bees are exposed to low doses of biocides for a period longer than 10 days, or even for the entire life span of the bees. Furthermore, the properties and mode of action of biocidal active substances may be similar or even identical to active substance of plant protection products. It was therefore decided to take over the “time reinforced toxicity” (TRT) assessment from the EFSA Bee guidance to the biocide assessment and to include it in the overall risk assessment strategy for bees (see Figure 3) in order to assess if the toxic effects of biocides at low doses over a long exposure period are higher than the effects at higher doses over a short exposure period (i.e., if toxic effects are reinforced by exposure time).

The TRT assessment is based on the extrapolation of the data coming from the standard 10-day chronic toxicity study for honey bees. The TRT assessment is divided into **two main parts** (Figure 14): the first part is the **hazard assessment** to estimate whether the biocidal active substance actually has a potential for time-reinforced toxicity, and the second part is the actual **risk assessment** for substances identified as having TRT properties in the first part. For the time being, the TRT assessment should only be performed for honey bees (for more information on this subject, see Annex G to the Supplementary Document (SD) of the EFSA Bee guidance).

The ECHA EG decided to focus the risk assessment for bees on active substances with an insecticidal mode of action and on biocidal products with relevant exposure to bees (see chapter 2 and section 6.1.2). Therefore, the hazard assessment, as the first part of the TRT assessment, must be carried out for those biocides that are relevant in terms of their risk to bees. The purpose of this part is to determine whether the biocide under consideration has TRT properties. When this is not the case, the standard chronic risk assessment as described in chapter 7 is considered sufficient to address the risk from long-term exposure. However, if the biocide shows TRT properties, the risk assessment, as the second part of the TRT assessment, is performed and the TRT assessment below will take precedence over the standard chronic risk case to estimate the overall predicted effect (i.e., the toxicity endpoints from the standard chronic risk case (LDD_{50} and slope) will be replaced by the TRT endpoints (i.e., $LDD_{50,TRT}$ and $slope_{TRT}$).

The TRT is an intrinsic property of an active substance. Therefore, the TRT assessment should always be performed for the active substances relevant in terms of their risk to bees as defined above. However, a chronic toxicity study with the biocidal product is also required if, based on acute data, the product was found to be more toxic compared to the active substance, or when

the biocidal product contains multiple active substances (see Table 42 in section 6.1.3). Whenever chronic data with the biocidal product is available, these data should be used in the hazard assessment of the TRT assessment, instead of the chronic data on the active substance. The worst-case chronic toxicity endpoints (i.e., $LDD_{50,TRT}$ and $slope_{TRT}$) between the results based on the product or the active substance data should be taken into account in the TRT hazard assessment.

In case of the presence of several active substances relevant for their risks to bees in a biocidal product, the TRT assessment should be performed for all active substances. If two or more active substances show TRT, a TRT risk assessment considering all these substances should be performed, using mixture toxicity. If only one active substance shows TRT, the TRT risk assessment is only to be done for this single substance.

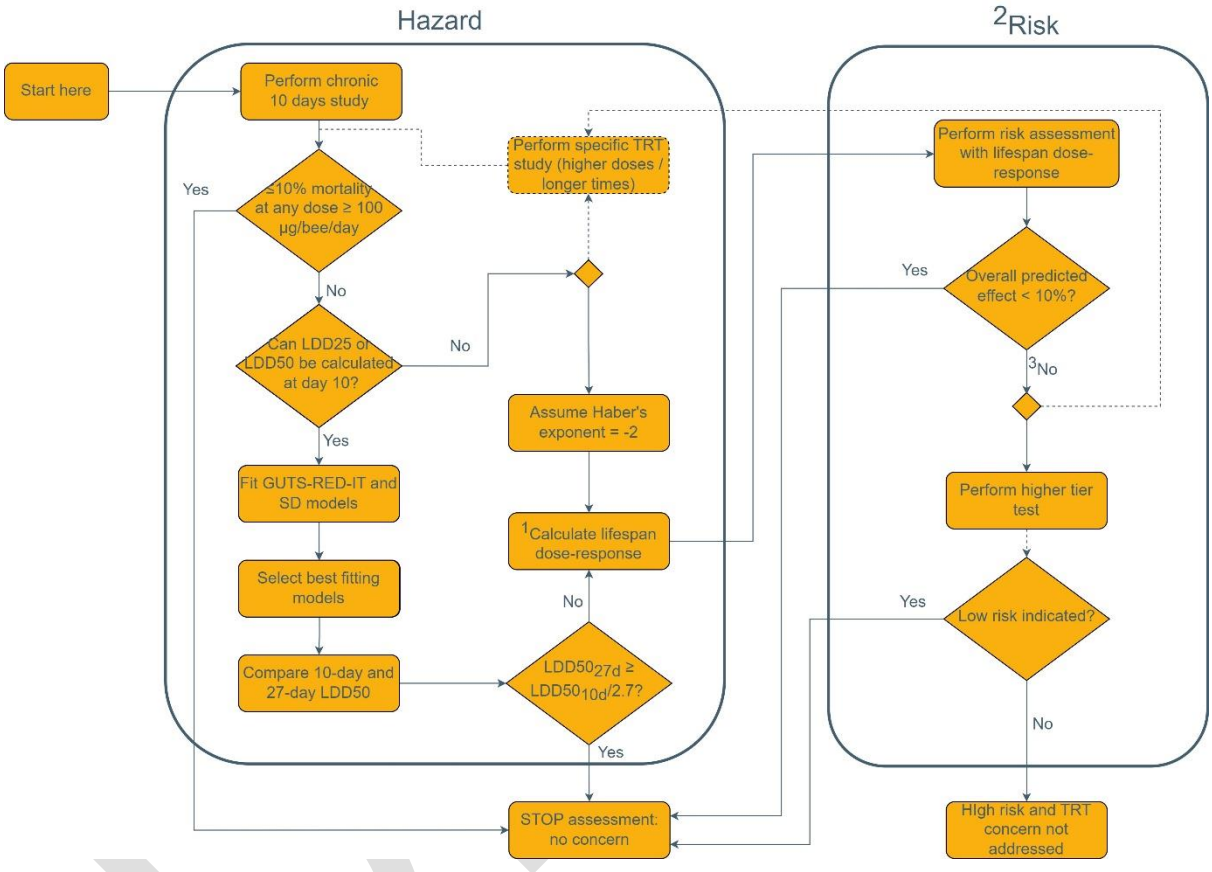


Figure 14: *Flowchart of the scheme for the assessment whether a substance exhibits time-reinforced toxicity*. (EFSA bee guidance)

Notes:
[1] Lifespan dose-response is calculated using the selected GUTS model (General Unified Threshold model of Survival), for both the active period and winter scenario
[2] This part has to be duplicated for the active period and the winter (inactive) period
[3] When an effect > 10% is predicted, either higher tier studies can be performed, or a specific TRT laboratory study can be executed. This second option is only applicable when this conclusion of >10% effect is reached on the basis of the worst-case assumption that Haber's exponent = -2.

8.1. TRT Hazard assessment

The hazard assessment part of the flowchart shown in Figure 14 intends to solve the question whether or not a biocidal active substance or product shows TRT. The steps to do so are described in section 8.1.1 below. If a substance is found to show TRT, a dose-response covering

the whole lifespan of a honey bee has to be determined, which will be used in the risk assessment part of the TRT assessment. The calculation of the lifespan dose-response is described in section 8.2.1.

8.1.1. Determining whether a substance shows time-reinforced toxicity

The hazard assessment is based on a methodology which relies on the use of GUTS (General Unified Threshold models of Survival)[13] TRT modelling. For a short introduction to the GUTS modelling framework, please refer to Section 4.4.1 of Annex G to the Supplementary Document of the EFSA Bee guidance. For further details on the background for the methodology used, please refer to Section 4.4.4 and 7.1.1 of that Annex G.

Step 1: check whether an assessment is necessary

The first step of the hazard assessment is based on the data of the standard 10-day chronic honey bee toxicity study according to the OECD test guideline 245 (OECD, 2017), which is required for the standard effect assessment for honey bees (see section 6.1.2). Therefore, the starting point is to collect data from the available study.

This first step consists in checking whether, in the 10-day toxicity study, the following conditions are met:

1. *Was the mortality ≤ 10% at any dose ≥ 100 µg/bee/day?*

   - *If yes, then a TRT assessment is not necessary*
   - *If no, go to step 2.*

This possibility to waive a further assessment for TRT was included to avoid unnecessary work and potential additional testing for substances of low toxicity to bees. The rationale for selecting the threshold of ≤ 10% mortality at any dose ≥ 100 µg/bee/day is described in Annex G to the Supplementary Document of the EFSA Bee guidance.

Note that if the highest dose tested in the available chronic 10-day test is below 100 µg/bee/day, step 1 of the scheme cannot be used. In that case, proceed to Step 2.

Step 2: check whether a robust GUTS-RED model can be fitted to the data

For a robust calibration of GUTS-RED models against the data from a 10-day chronic toxicity study, the level of mortality achieved at the end of the 10-day test period should be sufficient to allow calculation of an LDD_{50} or LDD_{25} value for at least day 10. If an LDD_{50} cannot be determined, an LDD_{25} can be considered instead. Therefore, the following condition should be met:

2. *Can a LDD_{25} or LDD_{50} be calculated at the end of the exposure period (day 10)?*

   - *If yes, fit both a GUTS-RED-IT and GUTS-RED-SD model to the data, and proceed to Step 3*
   - *If no, select one of the following options:*

     a. *Perform a new 10-day chronic toxicity study (according to OECD TG 245), using higher doses, and start again at step 1 using the newly obtained data*

[13] Several sources are available to download the GUTS modelling tools. The following website provides one of them: http://openguts.info/download.html

b. Perform a new chronic toxicity study, with a longer duration. Fit both a GUTS-RED-IT and GUTS-RED-SD model to the data, and proceed to Step 3

c. Assume the substance has TRT properties, with a worst-case Haber's exponent of -2. Calculate the lifespan dose-response as described in Section 7.1.2, ***Error! Reference source not found.****and proceed to the risk assessment.*

When ***option 2a****, which is to perform a* ***new chronic toxicity study using higher treatment doses,*** *is chosen, these doses have to be chosen so that the observed effects increase, and at least an* LDD_{25} *can be calculated at the end of the testing period. This should enable GUTS model fitting in Step 3a. Alternatively, when in the available study the highest tested dose was below 100 µg/bee/day, a new test including a dose of at least 100 µg/bee/day could be performed, so that this can be used in Step 1 and give the possibility to waive the TRT assessment if the mortality is ≤ 10% at dose ≥ 100 µg/bee/day. It is acknowledged that for substances of poor solubility, this might not be technically feasible using the technical active substance. In that case, performing a 10-day test (OECD TG 245) with the biocidal product instead of the technical active substance could be an option (as also proposed in Section 6.1.3). Alternatively, option 2b could be considered.*

In case of ***option 2b****, a* ***new chronic study with a longer duration*** *is performed. With the exception of the duration of the study, the study design should in this case also be based on OECD TG 245. To assess the validity of such a study, the validity criteria from OECD TG 245 also apply, at least to the data for the first 10 days of the test. For the time period beyond 10 days, additional validity criteria for the control are not considered necessary, as mortality in the control will inevitably increase over time. Note that the GUTS model, which is used in Step 3, is able to discriminate between background mortality and mortality from toxicant effects. Refer to Annex G to the SD of EFSA Bee guidance for some additional recommendations for the study design.*
Note that specific guidance on performing a study with longer duration life-long (also called 'life-long' or 'time-to-effect') test is currently not available, as this type of tests is still under development.

It should be noted that in the description of Step 2b above, it is assumed that it will be possible to use the data from the life-long test to fit a standard GUTS-RED model. It is expected that this will be possible in most cases. However, should the treatment-related mortality in the life-long test still be too low to enable a GUTS model fitting, this can be considered as an indication that TRT will not be an issue for that substance.

In case of ***option 2c****, it is* ***assumed that the substance has*** *TRT properties, with a worst-case* ***Haber's exponent of -2****.* An explanation on why this value of -2 is a worst-case, can be found in Section 7.1.1 of Annex G to the SD of the EFSA Bee guidance.

Step 3: Fit both GUTS-RED-IT and GUTS-RED-IT models to the data and select the best performing model
As it cannot be known a priori whether the GUTS-RED-SD or GUTS-RED-IT model will result in a better fit to the data, it is mandatory to use both models for TRT analysis. Both models can be fitted to the data using the currently available (semi-)automated calibration and prediction tools, thus generating the required output for the TRT assessment. The performance of both the fitted GUTS-RED-SD and GUTS-RED-IT model is compared based on the normalised root mean square error (NRMSE) for model calibration, and the model with the lower NRMSE value is the better performing one and should be selected over the other.

Note that in all standard GUTS implementations background mortality is described by a single parameter. While this is generally sufficient for 10-days test when the control mortality must remain below 15%, it may not be the case for life-long test, where background mortality is not

expected to be constant in time. In such case, a modified version of GUTS, which uses a 2-parameter model for control mortality has to be used.

Please refer to Section 5.1 of Annex G to the SD of the EFSA guidance for a detailed explanation of this modified version to be considered.

Step 4: Compare the 10-day and 27-day LDD_{50} and decide on TRT

As a final step to determine whether a biocidal substance or product shows TRT, the 10-day and 27-day LDD_{50} values should be derived from both the GUTS-RED-SD and GUTS-RED-IT model fitted to the data. If

$$LDD_{50,27d} \geq \frac{LDD_{50,10d}}{2.7}$$

then there is no concern about TRT. However, if this condition is not fulfilled i.e., $LDD_{50,27d}$ < $LDD_{50,10d}$ / 2.7, TRT cannot be excluded.

Since it is possible that the outcome is different between GUTS-RED-SD and GUTS-RED-IT, the following decision scheme should be followed to know on which model to base the conclusion:

- *If neither of the GUTS-RED-SD and -IT model does indicate TRT: it can be concluded that the substance does not show TRT. No further TRT risk assessment is required*
- *If both the GUTS-RED-SD and -IT model indicate TRT: it can be concluded that the substance shows TRT. Calculate the lifespan dose-response as described in Section 8.2.1, and proceed to the risk assessment.*
- *If one model indicates TRT and the other does not: use suggested metrics (NRMSE based on calibration data) to decide which of the two models to use.*
    - *If one model clearly fits better (shows lower NRMSE values), base the conclusion for TRT on the outcome from that model (i.e., compare the $LDD_{50,10d}$ and $LDD_{50,27d}$ derived from that model). Depending on the outcome, no further TRT risk assessment is required, or a lifespan dose-response as described in Section 8.2.1 needs to be calculated before proceeding to the risk assessment.*
    - *If there is no clear difference between both models, use the worst case (which will most likely be the SD model). In that case, it can be concluded that the biocidal active substance or product shows TRT. Calculate the lifespan dose-response as described in Section 8.2.1 and proceed to the risk assessment.*

8.2. Risk assessment based on TRT

For biocidal active substance or product for which there is concern for TRT following the steps described above of the hazard assessment, the standard chronic risk assessment (see Chapter 15.3) might underestimate the risk from long-term exposure. Therefore, for such substances, a specific risk assessment, which covers the whole lifespan of a bee, should be performed. This specific risk assessment supersedes the standard chronic risk assessment.

8.2.1. Calculating the lifespan dose-response (LDD_{50} and slope)

If a biocidal active substance or product is identified as showing TRT, or a worst-case approach assuming a Haber's exponent of -2 is followed, a risk assessment which covers the whole lifespan of a bee should be performed. In order to be able to perform such a risk assessment, the toxicity endpoint (LDD_{50} and slope) for a period of exposure that covers the whole lifespan should be known.

To estimate the toxicity endpoint for an exposure period corresponding to the lifespan of a honey bee, two scenarios are considered in the EFSA guidance: *a scenario that covers the active period of the bees (i.e., summer scenario), and a second that covers the inactive period of the bees (i.e., winter scenario*). A dose-response relationship for the whole lifespan should therefore be calculated for both scenarios, using a lifespan of 27 and 182 days for summer and winter bees (i.e., $LDD_{50,27d}$ and $slope_{27d}$, and $LDD_{50,182d}$ and $slope_{182d}$, respectively), as described respectively in Section 8.2.2 and 8.2.3.

GUTS-RED models fitted to the chronic toxicity data can be used to determine the dose-response at any timepoint. If in Step 3 of the workflow described in Section 8.1.1, one of the models (either GUTS-RED-IT or GUTS-RED-SD) was identified as better matching the data, the parameterization from the best model should be used to determine the 27- and 182-day dose-response. If there is no clear difference between both models, the one resulting in worst-case estimated 27- and 182-day dose-response should be used.

In case a worst-case approach assuming a Haber's exponent of -2 is followed, the linear C vs. t relationship (on a log-log scale) is used as a basis to calculate the lifespan dose-response. Since this option will likely be used in cases where the maximum effect is small (i.e., no reliable LDD_{25} can be obtained from the data), a surrogate 10-days dose-response can still be derived.

The input parameters for the TRT risk assessment to calculate the exposure for the two lifespan scenarios are discussed in the next sections 8.2.2. and 8.2.3.

8.2.2. Risk assessment for the active period

For the lifespan risk assessment during the active period, it is assumed that a honey bee will live for 27 days (see EFSA Bee guidance for more information on this value). *Given that the standard chronic risk assessment also focuses on bees during the active period, the same method for estimating the dietary exposure can be used in the lifespan risk assessment* (see Chapter 5.1). *The values for the different parameters* (e.g., Residue per unit dose of pollen/nectar (RUD) and DT50 in pollen and nectar, used to calculate the Predicted Concentration per Unit Dose in pollen/nectar (PCUD)), *as used in the standard risk assessment, can also be applied here.* However, there are two specific parameters for assessing the TRT of the active period:

- ***The time window for calculating time-weighted average concentrations (called "w")***, needed for the calculation of the PCUD. A time window of 27 days (corresponding to the median lifespan of an active honey bee) is used instead of 10 days (corresponding to the duration of a standard chronic oral toxicity study).

- ***Pollen and nectar consumption.*** *During their entire lifespan, honey bee workers undergo changes in their diet in relation to the tasks they execute. Thus, for this specific case, a combination of subsequent diets was considered. Specifically, it was assumed that bees perform nursing activities for 10 days (pollen and nectar consumption), then 8 days of additional in-hive tasks (nectar consumption similar to the nursing phase, no pollen consumption), and 9 days of foraging activity (higher nectar consumption due to flying activities, no pollen consumption).* See Section 5.3.4.5 of the Supplementary document of EFSA guidance for more details.

Taking the above parameters into account results in specific shortcut values, which are given in ECHA Bee guidance Appendix B. *The shortcut values are then used to estimate the dietary exposure PEQ for the active lifespan* (i.e., 27 days) with both the "above-soil contamination" and the "through soil contamination" exposure models. *As in the standard chronic risk assessment, the predicted individual level effect (PIE)* is then calculated using the active period lifespan PEQ and the $LDD_{50,27d}$ and $slope_{27d}$. *This is combined with the other three risk cases* (i.e., acute oral, acute contact and larvae; see section 7.1.3) *to estimate the overall predicted effect at the colony level.*

For the illustrative example (introduced in Section 7.2.1), referring to a hypothetical biocidal product applied to walls by spraying and assuming the substance shows TRT, example calculations for the active period are presented below. Since Vegetation margin exposure scenario is applicable for this kind of use and both above soil contamination and through soil contamination are relevant, the results are presented in two tables, Table 50 and Table 51, respectively. In this case, the dietary chronic predicted individual level effect is calculated based on the hazard parameters for the whole lifespan of a summer bee (27 days), and an exposure period of 27 days, instead of the 10 days as in the standard risk assessment for bees. For the other three risk cases and for the calculation of the PE_{SPG}, there are no differences from the standard risk assessment.

Table 50: Illustrative example on Tier 1 exposure assessment – Active period for a substance showing TRT properties (Dietary model for above soil contamination and contact).

Honey bees Tier-1 exposure (Dietary model for **above soil** contamination and contact)				
	Dietary			**Contact**
	Acute (da)	**Chronic (dc) (27 days)**	**Larvae (dl)**	**Acute (ca)**
Exposure PEQ_j [µg/bee] [a]	PEQ_{da} = 0.415	PEQ_{dc} = 0.092	PEQ_{dl} = 0.166	PEQ_{ca} = 0.658
Hazard parameters (DRCj): Dose-response model (Mod) Inflection point (e)[µg/bee][a] Slope parameter (b)	DRCda Mod: log-logistic e = 7 b = 1.84	DRCdc Mod: log-logistic e = **1.25** b = 1.67	DRCdl Mod: log-logistic e = 0.7 b = 2.24	DRCca Mod: log-logistic e = 15 b = 2.23
Step 1: Predicted individual level effect (PIE)	PIE_{da} = 0.5503%	PIE_{dc} = 1.27%	PIE_{dl} = 3.808%	PIE_{ca} = 0.0936%
Step 2: Predicted colony level effect (PCE)	PCE_{da} = 0.5503%	PCE_{dc} = 1.27%	PCE_{dl} = 3.808%	PCE_{ca} = 0.0936%
Step 3: combination of effects at colony level	$PE_{SPG} = 100 \cdot (1-(1-PCE_{da}/100) \cdot (1-PCE_{dc}/100) \cdot (1-PCE_{dl}/100) \cdot (1-PCEc_a/100))$ $= 100 \cdot (1-(1-0.005503) \cdot (1-0.0127) \cdot (1-0.03808) \cdot (1-0.000936))$ = **5.64%**			
PE_{SPG} i.e., ≤ 10%	Yes (acceptable risk identified)			

[a] Units are mentioned for brevity as µg/bee, but they are in fact µg/bee/day for chronic and µg/bee/dev. Period for larvae

Table 51: Illustrative example on Tier 1 exposure assessment - Active period for a substance showing TRT properties (Dietary model for through soil contamination and contact).

Honey bees Tier-1 exposure (Dietary model for **through soil** contamination and contact)				
	Dietary			**Contact**
	Acute (da)	**Chronic (dc) (27 days)**	**Larvae (dl)**	**Acute (ca)**
Exposure PEQ_j [µg/bee][a]	PEQ_{da} = 0.265	PEQ_{dc} = 0.164	PEQ_{dl} = 0.272	PEQ_{ca} = 0
Hazard parameters (DRCj): Dose-response model (Mod) Inflection point (e)[µg/bee][a] Slope parameter (b)	DRCda Mod: log-logistic e = 7 b = 1.84	DRCdc Mod: log-logistic e = **1.25** b = 1.67	DRCdl Mod: log-logistic e = 0.7 b = 2.24	DRCca Mod: log-logistic e = 15 b = 2.23
Step 1: Predicted individual level effect (PIE)	PIE_{da} = 0.24%	PIE_{dc} = 3.26%	PIE_{dl} = 10.74%	PIE_{ca} = 0.0%
Step 2: Predicted colony level effect (PCE)	PCE_{da} = 0.24%	PCE_{dc} = 3.26%	PCE_{dl} = 10.74%	PCE_{ca} = 0.0%

Step 3: combination of effects at colony level	$PE_{SPG} = 100 \cdot (1-(1-PCE_{da}/100) \cdot (1-PCE_{dc}/100) \cdot (1-PCE_{dl}/100) \cdot (1-PCEc_a/100))$ $= 100 \cdot (1-(1-0.0024) \cdot (1-0.0326) \cdot (1-0.1074) \cdot (1-0))$ $=$ **13.86%**
PE_{SPG} i.e., ≤ 10%	No (unacceptable risk identified)

[a] Units are mentioned for brevity as µg/bee, but they are in fact µg/bee/day for chronic and µg/bee/dev. Period for larvae

In the above example, the overall predicted effect at the colony level PE_{SPG} of 5.13% for above soil contamination is higher than what was predicted without consideration of TRT for the active period. Nevertheless, in this example, the SPG is not violated. However, the PE_{SPG} for the through soil contamination is 13.80%, which is above the acceptable threshold of 10% of effect on the colony. In fact, the TRT characteristics lead to more sensitive hazard parameters, thus to a lower LDD50 after 27 days, but also to lower exposure, since the relevant period is no longer 10 days but instead 27 days as a typical lifespan of a summer bee.

8.2.3. Risk assessment for winter bees

During the winter, honey bees will not forage for fresh pollen and nectar, but will feed on the food stored in the hive (i.e., honey). Therefore, the exposure of the bees to the biocides depends on the presence of residues in the honey. Given the differences in climatic conditions and agricultural and beekeeping practices in Europe, it is complicated to realistically estimate the extent of oral exposure of bees to contaminated honey. As a worst case and lower tier assessment, it is therefore assumed that the winter bees are fed 100% contaminated honey throughout the winter period (i.e., 182 days). For more information on the residues in honey and the lifespan of six months (i.e., 182 days), refer to Section 7.2.2 of Annex G to the Supplementary Document of the EFSA Bee guidance.

Assuming that winter bees feed on 100% contaminated honey, the dietary exposure is estimated consistently with the exposure models presented in Chapter 5. The specific parameters for the winter scenario needed for the TRT risk assessment of the inactive period are the following:

- ***Sugar consumption from honey****: bees consume* ***8.8 mg of sugar/day*** *in temperate regions, during winter to maintain the nest temperature at 5-8°C in the periphery and 15-20°C in the centre.*

- ***Sugar content in honey****: as the water content of honey is assumed to be 18%, the sugar content of honey would then be* ***82%****.*

- ***Dissipation rate in honey****: Given that there is currently no data available on the* DT_{50} *of active substances in honey, it was agreed to use a worst-case value for the lower tier risk assessment (i.e.,* ***1000 days****), which corresponds to no substantial dissipation.*

As for the risk assessment of the active period, the dietary exposure PEQ of the winter bees can be calculated either with the "above-soil contamination" and the "through soil contamination" model as presented below in sections 8.2.3.1 and 8.2.3.2, respectively. As in the standard chronic risk assessment, the predicted individual level effect (PIE) is then calculated using the winter period lifespan PEQ and the winter lifespan dose-response (i.e., $LDD_{50,182d}$ and $slope_{182d}$).

For the winter scenario, only chronic dietary exposure through honey consumption is considered. It is to be noted that the contamination in honey originates from nectar foraging only. Therefore, the winter bee scenario is relevant only if the exposed plants are attractive for nectar. In addition, as there are no larvae during winter, the dietary chronic PIE is calculated as a stand-alone assessment and the other risk cases (i.e., adult acute contact and dietary, and larvae) are not relevant for the winter scenario. The predicted individual level effect will correspond to the

overall predicted effects at the colony level (PCE).

8.2.3.1. Above-soil contamination

The following equation is used to calculate the PEQ due to dietary exposure for the winter bee scenario in case of relevant biocidal application (i.e., spraying on walls and foundation of houses, irrigation of gardens, large scale spray):

$$PEQ_{di} = \frac{AR}{1000}\ SV$$ **Equation 34**

Where: *AR = Application rate (g/ha)*
SV = shortcut value for dietary exposure through honey

The shortcut values are calculated using the following equation:

$$SV_{wi,above} = \frac{1}{1000}\ PCUD_{ho}\ \frac{CMP_{su,wi}}{S_{ho}}$$ **Equation 35**

Where:
- $CMP_{su,wi}$ *= 8.8 mg/day is the consumption of sugar in winter*
- S_{ho} *= 0.82 is the sugar content of honey*
- $PCUD_{ho}$ *= Predicted concentration per unit dose in honey (mg/kg)*
  = $RUD_{ho}\ \frac{1-e^{-kw}}{kw}$ *, with k= ln(2)/DT_{50}, DT_{50} in honey = 1000 days (if no data available on dissipation in honey) and w = 182 days.*

For more information on the database for RUD values in honey, refer to the Appendix B in Annex G to the Supplementary Document of the EFSA Bee guidance. *Based on this database, a 90th percentile RUD for residues in honey of 3.0 mg/kg was derived.* Shortcut values are presented in Table 52. Following from the illustrative example (introduced in Section 7.2.1), an example calculation for winter bees is given for above soil contamination in Table 53 for a substance that shows TRT.

Table 52: Shortcut values for dietary exposure through honey (winter bees) for above soil contamination

Application	**Shortcut value (µg/bee/day) For crop/grass/treated plant on the treated area**	**Shortcut value (µg/bee/day) For mixed vegetation**
Spray applications	0.03	0.015

Table 53: Illustrative example on Tier 1 exposure assessment – Winter bees for a substance showing TRT properties (Dietary model for above soil contamination and contact).

Honey bees Tier-1 exposure (Dietary model for **above soil** contamination and contact)					
		Dietary			**Contact**
		Acute (da)	**Chronic (dc) (182 days)**	**Larvae (dl)**	**Acute (ca)**
Exposure	PEQ_j [µg/bee] [a]		PEQ_{dc} = **0.009**		
Hazard parameters (DRCj): Dose-response model (Mod) Inflection point (e)[µg/bee][a]			DRCdc Mod: log-logistic e = **1.25**		

Slope parameter (b)		b = 1.67		
Step 1: Predicted individual level effect (PIE)		PIE_{dc} = 0.025%		
Step 2: Predicted colony level effect (PCE)		PCE_{dc} = 0.025%		
Step 3: combination of effects at colony level	$PE_{SPG} = 100 \cdot (1- (1-PCE_{dc}/100))$ $= 100 \cdot (1-(1-0.00025))$ $=$ **0.025%**			
PE_{SPG} i.e., ≤ 10%	Yes (acceptable risk identified)			

[a] Units are mentioned for brevity as µg/bee, but they are in fact µg/bee/day for chronic and µg/bee/dev. Period for larvae

8.2.3.2. Through soil contamination

Consistently with what was agreed for the above-soil contamination model, the 90th percentile value from the database for RUD values in honey (3.0 mg/kg) is considered for through soil contamination as well. As presented in Section 5.1.1., residues in nectar for contamination via soil can be estimated by using PEC_{pw}. The same is considered to be true for residues in honey. Thus, the following equation is used to calculate the PEQ due to dietary exposure for the winter bee scenario in case of relevant biocidal applications (i.e., manure/sewage sludge application on soil, spraying on walls and foundation of houses and irrigation of gardens).

$$PEQ_{di} = SV_{wi,so} = \frac{1}{1000} \times PEC_{pw} \times \frac{CMP_{su,wi}}{S_{ho}} \times \frac{1 - e^{-kw}}{kw}$$ **Equation 36**

Where: PEC_{pw} *= Predicted Environmental Concentration in pore water (mg/L = mg/kg)*
$CMP_{su,wi}$ *= 8.8 mg/day is the consumption of sugar in winter*
S_{ho} *= 0.82 is the sugar content of honey*
k *= ln(2)/DT_{50} and w = 182 days.*

In the Tier 1 of the exposure assessment, the PEC_{pw} is assumed to be 1 mg/kg, for any application regime where the cumulative application rate is not higher than 4.5 kg/ha. In cases where the cumulative application rate is higher than 4.5 kg/ha, Tier 2 exposure estimation have to be conducted by refining PEC_{pw}, as described in chapter 5.

Note that all parameters used for Tier 1 exposure estimations are in this case fixed, therefore, the exposure estimation for winter scenario and through soil contamination model is 0.011 *µg/bee/day.*

Table 54: Shortcut values for dietary exposure through honey (winter bees) for through soil contamination.

	Shortcut value (µg/bee/day) For crop/grass/treated plant on the treated area	**Shortcut value (µg/bee/day) For mixed vegetation**
Through soil contamination	0.011	0.0055

Table 55: Illustrative example on Tier 1 exposure assessment – Winter bees for a substance showing TRT properties (Dietary model for through soil contamination and contact).

Honey bees Tier-1 exposure (Dietary model for **through soil** contamination and contact)

		Dietary			Contact
		Acute (da)	**Chronic (dc) (182 days)**	**Larvae (dl)**	**Acute (ca)**
Exposure	PEQ_j [µg/bee]		PEQ_{dc} = **0.0055**		
Hazard parameters (DRCj): Dose-response model (Mod) Inflection point (e)[µg/bee]a Slope parameter (b)			DRCdc Mod: log-logistic e = **1.25** b = 1.67		
Step 1: Predicted individual level effect (PIE)			PIE_{dc} = 0.012%		
Step 2: Predicted colony level effect (PCE)			PCE_{dc} = 0.012%		
Step 3: combination of effects at colony level		$PE_{SPG} = 100 \cdot (1- (1-PCE_{dc}/100))$ $= 100 \cdot (1-(1-0.00012))$ = **0.012%**			
PE_{SPG} i.e., ≤ 10%		Yes (acceptable risk identified)			

In case of the illustrative example of the hypothetical biocidal product applied to walls by spraying, where release processes contribute to both, above soil contamination and through soil contamination, the overall predicted effect at the colony level on winter bees PE_{SPG} is 0.025% and 0.012% for the above soil and the through soil contamination, respectively (Table 53 and Table 55). The risk is therefore acceptable. The PEQdc value of 0.0055 mg/bee/day corresponds to the shortcut value for the mixed vegetation, which is relevant for biocide exposure.

8.2.4. Refinement options

If the Tier 1 risk assessment for the active period and/or the winter bee scenario shows high risk (i.e. the SPG is not met), it is possible to refine either some parameters of the exposure equations, as can be done for the standard risk assessment (see chapter 5*), or to perform a specific TRT study, as also described in Section 8.1.1.* (step 2 of the hazard assessment*). The latter would especially be useful for those* substances for which an LDD_{50} or LDD_{25} cannot be determined, and *for which it could therefore be assumed a worst-case Haber's exponent of -2 for calculating the lifespan dose-response.*

*For the lifespan risk assessment for the **active period**,* the dietary exposure estimations can be refined using all options presented for the standard risk assessment (see sections 5.2 to 5.7).

*For the lifespan risk assessment for the **winter bees**, the only two parameters in the dietary exposure model that could be refined using substance-specific data, are the residues in honey and the DT_{50} in honey*. However, data on the residues in honey is usually not available in the biocide dossier. For further information on the refinement of residues in honey, refer to section 7.2.3 of Annex G to the Supplementary Document of the EFSA Bee guidance. Eventually, a refinement of the PEC_{pw} could be possible, as presented in sections 5.2 to 5.7.

The lifespan of a winter bee of 182 days is a very rough and conservative estimate. In theory, this value could be refined if more detailed data would be available. However, it should be noted that any refinement of the winter bee lifespan would have only a rather minor effect on the estimated lifespan-LDD_{50} (see Annex G to the Supplementary Document of the EFSA Bee guidance for details). Therefore, this kind of (general) refinement is not considered very useful, unless the outcome of the risk assessment is borderline or it can be demonstrated that the length of the winter period is substantially less than three months, which is hardly the case for Europe.

Chronic toxicity data can further be refined by conducting higher tier effect field studies (see Chapter 10). *Generally, these requirements are the same for substances that show time-reinforced toxicity and those substances that do not. However, for substances with TRT properties, a field study must be sufficiently long to ensure that potential effects following long-term exposure are taken into account. In practice, this means that the study should not be started later than September and last until next spring, thus including overwintering and*

observing the honey bees for at least half a year. This type of study designed for plant protection products is challenging and may not be technically feasible for biocides. Therefore, refinement of chronic toxicity data might be a difficult option for biocides. Nevertheless, any available higher tier data could be evaluated on a case-by-case basis.

9. Sublethal effects on honey bees in risk assessment

9.1. Overall strategy

Sublethal effects result from an exposure dose that does not directly cause death. Therefore, it is difficult to establish a direct link between the sublethal effects observed in a standard study and the strength of the honey bee colony (see definition of SPGs). Nevertheless, a wide range of adverse sublethal effects on behaviour, physiology, longevity, or reproduction have been reported for many bee species in the open literature (see Annex K to the Supplementary Document of the EFSA Bee guidance). Thus, due to growing concerns and specific requests for greater consideration of sublethal effects in the risk assessment of pesticides for bees, it was decided to include the assessment of sublethal effects in the EFSA Bee guidance. For the same reasons, the ECHA EG also decided to consider, in parallel to the TRT assessment, the sublethal effects in the risk assessment of biocides for bees (see Figure 3). Note that if the lower tier assessment were to lead to a higher tier assessment with higher tier studies, it is assumed that the sublethal effects could be covered by these studies and there would be no need to assess sublethal effects in addition. However, if the standard lower tier risk assessment, based on mortality endpoints, shows low risk, the sublethal effect assessment should be performed.

As the spectrum of observed sublethal effects is wide and there is a lack of standardisation of studies to assess these effects, it was decided for the EFSA Bee guidance to focus on sublethal effects that may alter bee behaviour, in particular feeding and foraging behaviour. Indeed, it is assumed that a significant change in the diet of a colony, caused by a significant alteration in foraging, can indirectly have a negative impact on the colony strength. In addition, it is to be noted that observations of feeding behaviour are already included in the OECD test guidelines of the standard laboratory studies that are required for standard risk assessment. This could allow potential risks related to sublethal effects to be identified quite easily without the need for further lower or higher tier studies. Nevertheless, if a concern of sublethal effects were to be raised, it could still be further investigated in specific higher tier studies (see Section 9.3). Furthermore, as most of the standard tests are carried out and most of the data is available for honeybees, it was also decided to limit the assessment of sublethal effects to honeybees.

Sublethal effects are strongly linked to the mode of action of a chemical. As the ECHA Bee guidance will focus primarily on insecticidal substances/products (see Section 2.1) and various sublethal effects related to the insecticidal mode of action have already been reported in the open literature (see also Annex K to the Supplementary Document of the EFSA Bee guidance), the assessment of sublethal effects, in parallel to the lower tier assessment, is justified and is also required for the risk assessment of biocides for bees. However, as the link between sublethal effects on foraging behaviour and colony strength is still to be confirmed, the outcome of the sublethal effect assessment, as described below, can therefore only be "concern for sublethal effects indicated" or "no concern for sublethal effects indicated". In addition, it is expected that further recommendations and improvements of the approach can be provided as experience is gained.

9.2. Strategy for identifying concern for sublethal effects from lower tier information on honey bees

In the EFSA Bee guidance, a strategy for assessing sublethal effects is proposed and is presented in Figure 15 below. Further information to the overall strategy is also available in Chapter 9 of

the Supplementary Document to the EFSA Bee guidance.

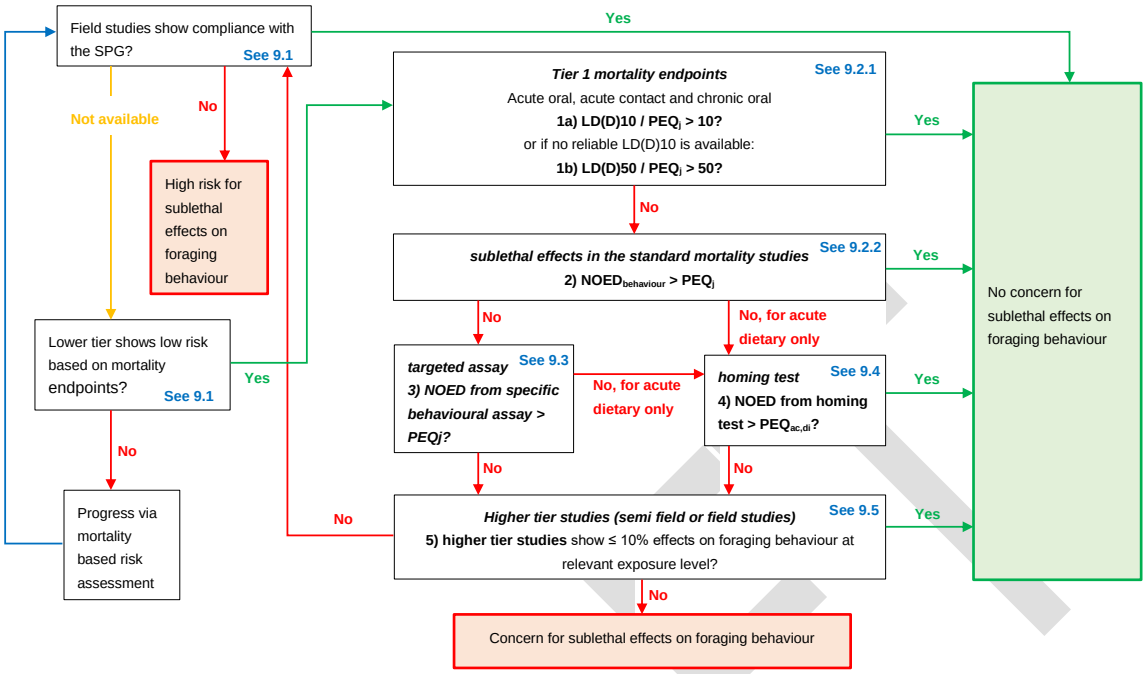


Figure 15: Assessment strategy for sublethal effects. SPG = Specific Protection Goal; LDD = Median Lethal Dietary Dose; PEQ = Predicted Exposure Quantity, where *j* denotes the risk case and *ac,di* refers to acute, dietary; NOED = No Observed Effect Dose

9.2.1. Toxicity/exposure ratio using mortality endpoints

The first step in the assessment of sublethal effects is to estimate the "level of no concern", i.e., a sufficiently low exposure (i.e., PEQ_j) at which no effect on foraging behaviour is expected. The "level of no concern" can be calculated as the LD(D)10 divided by 10, evaluated separately from the standard laboratory tests on honey bees (i.e., acute oral, acute contact and chronic oral) (for more details on the toxicity/exposure ratio calculation, see Section 9.2.1 to the Supplementary Document of the EFSA Bee guidance). If a reliable LD(D)10 cannot be calculated, the LD(D)50 divided by 50 can be used, which would be a worst case "no concern level". The "level of no concern" is then compared to the corresponding PEQ. A concern for sublethal effects is triggered if:

- *1a)* ***PEQ_j > LD(D)10 / 10, i.e., L(D)D10 / PEQj > 10,***
  or if no reliable L(D)D10,

- *1b)* ***PEQ_j > LD(D)50 / 50, i.e., L(D)D50 / PEQj > 50***

Where:

- *the PEQ_j (j indicates different PEQ values for the relevant risk cases) values are calculated according to Chapter 5. The most refined PEQ_j available can be used.*
- *For limit tests in which no significant mortality is observed, the following stands: LD(D)50 = LD(D)10 = NOED, meaning that the case 1a) is applicable.*
- If TRT properties are determined, the lifespan LDD50 or data from the specific TRT study should be used. *Since foraging behaviour is only relevant for summer bees, the 27-d LD(D)50 and corresponding PEQj should be used (see* ***Error! Reference source not found.****Chapter 8 for more information).*

If the PEQ_j is lower than the 'no concern level' (1a, 1b) for each of the three standard toxicity

studies on honey bees, i.e., acute contact, acute dietary and chronic dietary, then no concern for adverse effect for foraging behaviour can be concluded and no more consideration is needed. If the PEQ_j is higher than the 'no concern level' a potential concern is identified, and the risk assessor should consider the next step (step 2 in 9.2.2).

9.2.2. Using pattern of sublethal effects seen in the laboratory tests

The second step consists in measuring, again on the basis of standard toxicity test data, the abnormal feeding behaviour of bees and the amount of food consumed. *For this purpose, it is proposed in the EFSA Bee guidance to use the regular observations required in the OECD test guidelines 213, 214 and 245.* From these behavioural observations in standard laboratory tests, it should be possible to determine if exposure to biocides influences the behaviour of bees in laboratory experiments (i.e., determine the `no concern level´ $NOED_{behaviour}$). *Further standardisation can be achieved by following the recommendations in the supplementary materials of Tosi and Nieh (2019).* For more information regarding the reliability of the data, refer to Section 9.2.2 of EFSA Bee guidance.

Sections 9.2.2.1 to 9.2.2.3 of the EFSA Bee guidance explain how to derive a $NOED_{behaviour}$ value for abnormal bee behaviour from the standard laboratory tests.

There is no concern indicated from the sublethal effects on foraging behaviour if:

- 2) **the $NOED_{behaviour}$ > PEQ_j**

If this is not the case, additional studies are needed, see step 3 in Section 9.3 and step 4 in Section 9.4.

As no direct link can be established between the behaviour of bees in a laboratory context and mortality, *any statistically significant difference in behaviour between the treatment and control groups should be treated as indicative of a potentially important sublethal effect that requires further investigation*.

9.2.2.1. Statistical analysis of behavioural effects to derive 'no concern level' ($NOED_{behaviour}$)

Ideally, the appropriate statistical model should be able to describe not only the dose-response trend, which is the focus of this analysis, but also the temporal pattern of abnormal observations. This may be problematic, however, as individuals showing behavioural abnormalities may revert to normal behaviour over time, which may result in the absence of a clear and consistent trend over time, in contrast for mortality studies where the trend is, by definition, increasing. Accounting for the temporal pattern may thus hinder the analysis and produce results which are difficult to interpret. Therefore, it is recommended in the EFSA Bee guidance to analyse the data aggregated across the experimental period. A single aggregate proportion should be calculated for each cage (the sum of daily observations of abnormal behaviour divided by the sum of the daily count of live bees). The analysis should then focus on investigating whether the aggregate proportions show an increasing trend with dose levels.

OECD recommendations (OECD, 2006) have been followed to use a statistical test for trend combined with a 'step-down' procedure. This test is focussed on the detection of a monotonic (increasing) trend and should be appropriately selected among the range of options presented in OECD (2006). A good choice, to provide an example, could be the Rao-Scott adjusted Cochran-Armitage test (RSCA) (Rao and Scott, 1992), which has several desirable advantages: it is generally the most robust choice for quantal data (proportions), it allows for overdispersion, and it takes experimental replication into account (Green et al., 2018).

An appropriate statistical test for trend is chosen and the data is analysed using a step-down procedure following the method described in OECD (2006), meaning:

- *The test for trend should be performed for data from all the treatment groups including the control. The cages should be incorporated as subgroups (or clusters) in the test.*
- *If the test is significant (α = 0.05) then there is an increasing response across all dose levels. The high dose group is omitted and the test for trend is repeated with the remaining dose groups.*
- *The procedure is continued until the test is non-significant – there is no increasing response across the remaining dose groups. The highest concentration remaining at this stage is the NOEC.*

9.2.2.2. 9.2.2.3Statistical analysis of behavioural effects to derive the 'no concern level' for food consumption ($NOED_{behaviour,food}$)

In acute and chronic dietary studies, the applicant should also test to see if the biocides induce changes in food consumption. The analysis should be based on an analysis of variance and include a dose, day, and day by dose interaction in order to be able to compare the mean volume of food consumed between each treatment group and the control. The comparison should be done for each day; a Bonferroni-Holm correction for multiple comparisons can be used.

9.2.2.3. Worked example of how to analyse the behavioural observations after exposure to a biocide.

The aim of this example is to demonstrate the calculation of a NOEC from data taken from an anonymised plant protection product dossier study. The data consists of a 10-day chronic exposure study where the number of abnormal behaviours, as described in OECD test guideline 245, were recorded every 24 hours. The data is presented in Figure 16 *below and was analysed using a RSCA test with a step-down procedure, as described in Section 9.2.2.1. The model returned significant p-values for the first four steps (all p < 0.001) indicating that each of the four highest concentrations of the product had a higher proportion of abnormal behavioural observations than the control. When the model included only the control and the lowest tested concentration of the product, 156.25 mg a.s./kg, the trend was not significant (p = 0.483), indicating that this can be considered as the NOEC value from this experiment. In the 156.25 mg a.s./kg treatment group, the accumulated mean uptake of the test item was 43.99 µg a.s./bee. Thus, the interpretation of this test would be that a $PEQ_{di,ch}$ of <43.99 µg as/bee would not trigger a concern for sublethal behaviour, a $PEQ_{di,ch}$ ≥156.25 µg a.s./bee would trigger a concern for sublethal effects and the applicant should proceed to further targeted behavioural tests (see steps 3 and 4 below).*

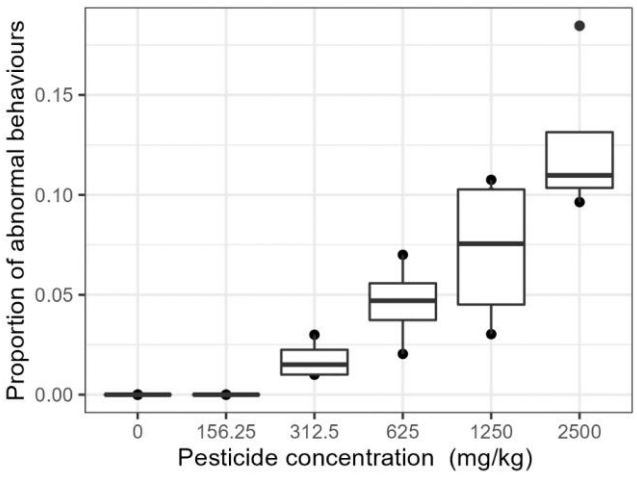


Figure 16: ***Boxplot showing the proportion of abnormal behaviour across a range of plant protection product concentrations aggregated across the entire experiment, note that the y axis is truncated at 0.2.*** **(EFSA Bee**

guidance)

9.3. Specific behavioural assays on honey bees

As a third step, if a potential concern is raised on the basis of abnormal behavioural observations in the standard laboratory studies, targeted behavioural tests can be carried out. These studies can be similar to the standard toxicity tests, but performed at lower doses, while ensuring that the *predicted exposure of the exposure assessment tiers is covered in the test. In addition, these studies should implement the following modifications to improve the data quality on sublethal effects:*

- *Even with training, interpreting the behaviour of an animal can be subjective. In order to minimise any unconscious bias, it is necessary that all behavioural results are generated blind (i.e. the observer does not know which treatment was given to which group).*

- *The number of replicates should be increased. The applicant has to demonstrate that the experiment is large enough to detect an effect size of at least 10% more observations of abnormal behaviour in the treatment group relative to the control group with an alpha of 0.05 and a power of 0.8.*

- *Further standardisation of behavioural assessments can be achieved with the recommendations in the supplementary materials of Tosi and Nieh (2019).*

In addition, it has to be noted that using the OECD design, the behavioural observations can only be monitored as the proportion of individuals behaving abnormally at any point, making the unit of replication the cage. If individual bees can be marked, either by using paints or identification tags, then the behaviour of each individual can be recorded at each timepoint, potentially making the unit of replication the individual and increasing the statistical power of the test to detect an effect. This is however considered a potential future improvement, not implementable at present.

The modifications mentioned above (blind observer and increased number of replicates) can also be implemented directly in the mortality tests. It is recommended to consider these in future modifications of the OECD guidelines for acute and chronic adult bee toxicity tests.

The $NOED_{behaviour}$ from the targeted assay should be compared to the relevant exposure level.

There is no concern indicated from sublethal effects on foraging behaviour if:

- 3) ***the $NOED_{behaviour}$ from targeted assays > PEQ_j***

9.4. Homing flight study

The Annex K of the Supplementary Document of EFSA Bee guidance provides an overview of possible tests to investigate sublethal effect, some of which have a behavioural trait as an endpoint. Most of them are not standardized tests, except the homing flight study (OECD guidance document 332, 2021). This study aims at assessing effects of acute oral exposure to sublethal doses of a substance on the homing flight of worker honey bees. As the study only assess the effect of acute exposure, it can only be used as a refinement of a concern identified from the acute exposure. The dose level in the test needs to cover the acute daily intake of a forager bee.

From the data in this study, a $NOED_{homing\ test}$ can be determined. As mentioned above for other non-standardised targeted behavioural tests, there is no concern indicated from acute sublethal effects on foraging behaviour if:

- 4) ***$NOED_{homing\ test} > PEQ_{ac,di}$***

9.5. Higher tier endpoints

The last step gives the possibility to use data from higher tier studies in cases where they are available, but not reliable enough to perform a higher tier risk assessment. As mentioned in Section 9.1, should a higher tier assessment with higher tier studies be available or be carried out for a biocide, it will cover the risks of sublethal effects.

However, it is possible to assess effects on foraging behaviour in these higher level studies to obtain additional information on the mode of action of biocides. In this case, a negative effect on foraging behaviour can be determined if there is a 10% reduction compared to the untreated control in one or more of the following parameters:

- The amount of pollen collected per flight (in mass)
- The number of bees returning with pollen
- The duration of a foraging flight (in minutes/flight)

More details on the assessment of these parameters can be found in the EFSA Bee guidance Section 9.5.

- 5) ***If no effect >10% was seen at this exposure level, there is no concern from sublethal effects on foraging behaviour.***

10. Higher tier risk assessment

Higher tier risk assessment may be triggered when an unacceptable risk is identified in the lower tier risk assessment. The goal of the refinement is to reduce uncertainty through increased amount of information from studies conducted under more representative environmental conditions than standard laboratory tests. Regarding honey bees, the objective of the higher tier assessment is to check whether the agreed SPG is met (similar to lower tier risk assessment). For bumble bees and solitary bees, no SPG is currently defined due to current absence of knowledge and thus comparison of higher tier risk assessment output to SPG is not possible. Until the SPGs for bumble bees and solitary bees are defined, the EFSA Bee guidance advices to require more frequently higher tier studies to allow better protection of these bee species.

In the EFSA Bee guidance, three types of higher tier effect studies are described: field studies, semi-field studies and colony feeder studies (Table 56). The EFSA Bee guidance provides recommendations for the circumstances when each study type would be useful in relation to the outcome of the lower tier risk assessment (EFSA Bee guidance Section 3.3, Figure 4 and Section 10). In higher tier studies, it is necessary to measure the concentration of residues in pollen and nectar to verify the actual exposure levels.

Table 56: Overview of the higher tier study types for honey bees presented in the EFSA Bee guidance.

Study (HB)	Description
Field study	Colonies with free flying bees are studied in open field conditions in (agricultural) landscapes. Colony size is the endpoint used for statistical comparison.
Semi-field	Bee colonies enclosed in large cages in field conditions. Forager mortality and foraging behaviour are the endpoints

	used for statistical comparison.
Colony feeder	Free flying colonies in open field conditions but with limited food (spiked sugar solution). The number of covered brood cells is the endpoint used for statistical comparison.

In the case of biocides, the performance of a higher tier risk assessment would be dependent on the case since these studies designed for plant protection products are challenging and may not be technically feasible for biocides. It has therefore been considered that higher tier field studies for plant protection products may not be directly applicable for biocidal products[14]. However, if the design is adapted, the tests may be applicable to biocide uses as well. For instance, semi-field studies could be relevant for biocidal products if an unacceptable risk is identified in the lower tier risk assessment.

It is advised for the applicant to consult the corresponding section of the EFSA Bee guidance (Chapter 10), and to discuss with the evaluating competent authority, before performing any type of higher tier studies to ensure that an adequate testing protocol will be applied. In addition to the description of the aim, methodology, main considerations, and the endpoints for each higher tier study type, the EFSA Bee guidance provides instructions for a weight of evidence and uncertainty analysis for the use of the studies in the higher tier risk assessment. Under the biocide risk assessment, any available higher tier data needs to be evaluated on a case-by-case basis.

11. Metabolite assessment

11.1. Method

For biocides, an environmental risk assessment should be performed for active substances, as well as their metabolites (as stated in BPR Annex VI art 8, 32 and 73, in Introduction to guidance on the Biocidal Products Regulation, Part A: Information requirements, Volumes I – IV, Chapter 4 (ECHA, 2022), and in Guidance on the Biocidal Products Regulation, Volume IV, B+C, Section 2.1 and further (ECHA, 2017)). Metabolites trigger concern for bees when they are identified in plant materials like pollen, nectar, or other attractive plant matrices. For biocide applications, assessment of metabolites in these plant materials is not a standard practice. BPR Annex II includes information requirements for biodegradation and transformation in water, manure, soil, and air. However, the information requirement in BPR Annex II 10.4 to support the approval of an active substance states 'Additional studies on fate and behaviour in the environment'. This includes any relevant environmental compartment or matrix. Hence, when bees are expected to be exposed (see Chapters 4 and 5) and a risk assessment is triggered, data measuring the residues and metabolization processes of the active substance and its degradation products in the relevant matrices are required. As experience with the generation of relevant metabolite data in plant matrices is very limited for biocides and the risk assessment for the active substance covers the metabolites in many cases (refer to EFSA Bee guidance, Appendix C), a stepwise approach depending on the source of exposure/scale is used for the metabolite risk assessment.

As a first step, the metabolite risk assessment will only be required for larger scale applications,

[14]Minutes of the 92nd meeting of representatives of Members States Competent Authorities for the implementation of Regulation (EU) No 528/2012 concerning the making available on the market and use of biocidal products, available at https://health.ec.europa.eu/events/92nd-meeting-expert-group-implementation-biocidal-products-regulation_en

including large scale spraying, irrigation, and manure/sewage sludge applications (see Chapter 5). Residue data and metabolism studies in nectar, pollen, and plants with the active substance and its possible metabolites are required only if it is anticipated that the substance is used in products that are sprayed outdoors (e.g., large scale spraying and irrigation). For through-soil exposure (manure/sewage sludge application) a different approach is taken, using data which are more generally available for active substances under the BPR and more easily obtained for biocidal uses. In this approach, metabolites formed in soil are assessed and this information can be obtained from the assessment for the terrestrial compartment. Refer to Figure 17 for a decision tree for the metabolite assessment for biocidal uses, and to the paragraphs below for further information on the assessment for large scale spraying, irrigation, and manure/sewage sludge applications.

11.1.1. Assessment for large scale spraying and irrigation

Since typically data on plant residues and metabolism are not available in the biocide dossiers, a non-test screening step is introduced in the assessment of large scale spraying and irrigation applications. For this screening effect tier, no further metabolite assessment is needed if the risk for the (parent) active substance is unacceptable. In addition, in the screening step no further data are initially required for metabolites if the risk for the active substance is acceptable. Instead, if an acceptable risk was identified in the risk assessment of the (parent) active substance, then a screening for any potential metabolites should be performed, assuming a 10-times higher toxicity compared to the parent. In practise, this means that the standard risk assessment is re-run with the 10-times more toxic effect values. For this screening effect tier, no further data are required for metabolites if the risk for the metabolite is acceptable (i.e., PE_{SPG} ≤ 10%) with the 10-times higher toxicity. If using this approach, the risk is unacceptable, further data are required on metabolites.

When the screening indicates an unacceptable risk for potential metabolites, an assessment should be performed in line with the EFSA Bee guidance, for large scale outdoor spraying and irrigation. Applicants should contact the evaluating competent authority prior to the start of any study and prior to the submission of a dossier containing studies on metabolites. In order to select the most appropriate testing strategy, the problem formulation for metabolite assessment should be considered in light of the outcome of the lower tier risk assessment, relevant exposure pathway(s) and plant species. For information on residue data, refer to the EFSA Bee guidance Appendix C, however for biocides the exposed plants in most cases are of an unknown and mixed composition. If one plant species is treated with the biocide, then this species should be included in the residue/plant metabolism studies. If a mixture of plant species may be exposed to the biocide, a surrogate species should be included in the studies. In line with the EFSA Bee guidance (Appendix C), the submission of a residue trial performed in a surrogate flowering species (for example Phacelia, oilseed rape, or sunflowers) may be considered for the purpose of identification of relevant metabolites in pollen and nectar.

A risk assessment for metabolites identified in the available studies (e.g., plant metabolism studies) is triggered when:

- residues of metabolites are found at or above 10% TRR (Total Radioactive Residue) **and** 0.01 mg eq/kg (OECD, 2007) in residue studies in pollen and nectar or metabolism studies in treated plants,

OR

- residues of metabolites are found at or above 10% TRR (Total Radioactive Residue) **or** 0.01 mg eq/kg in residue studies in pollen and nectar or metabolism studies in treated plants, **and** their parent substance is of acute toxicity to bees (i.e., LD50 < 0.01 µg/bee).

When a metabolite requires further assessment, based on the criteria above, relevant information on the hazard and exposure of the metabolite to bees must be provided. The

assessment should be in line with the EFSA Bee guidance Chapter 11, following the exposure assessment approaches for biocide sources of exposure detailed in Chapter 5).

11.1.2. Assessment for manure/sewage sludge application

A risk assessment for metabolites is triggered when:

- metabolites in soil/porewater formed ≥ 10% on a molar basis, of the active substance in soil / porewater or appearing at two consecutive sampling points at amounts ≥ 5% on a molar basis, or if at the end of the study the maximum of formation is not yet reached but accounts for ≥ 5% on a molar basis, of the active substance at the final time point (in line with Guidance on BPR: Vol IV ENV Parts B+C), **and**

- their parent substance is of acute toxicity to bees (i.e., LD50 < 0.01 µg/bee).

As a first step, screening can be performed for the relevant soil metabolite(s), assuming a 10-times higher toxicity compared to the parent. For this screening effect tier, no further data are required for metabolites if the risk is acceptable (i.e., PE_{SPG} ≤ 10%). If the risk is unacceptable on the basis of first tier porewater calculations, refinement of the porewater concentration can be derived (PEARL groundwater calculations). If on the basis of these calculations an unacceptable risk is still identified, further data are required investigating the uptake characteristics of these metabolites in plants, pollen and nectar and/or the toxicity of the relevant metabolite(s) to bees. Applicants should contact the evaluating competent authority prior to the start of any study and prior to the submission of a dossier containing such studies on metabolites. In order to select the most appropriate testing strategy, the problem formulation for metabolite assessment should be considered in light of the outcome of the lower tier risk assessment, relevant exposure pathway(s) and plant species.

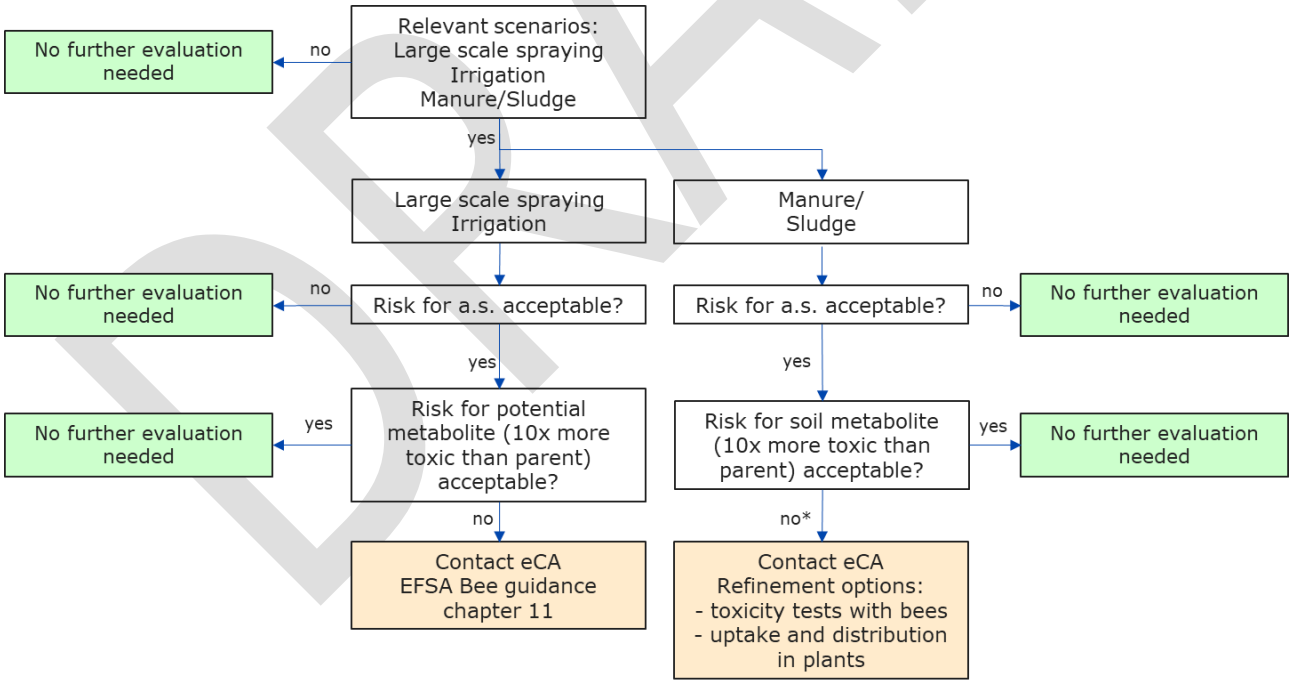


Figure 17: Decision tree for the metabolite risk assessment for biocides. *If in the risk for metabolites for manure/sewage sludge application is unacceptable, refinement of the porewater concentration can be derived.

eCA = evaluating competent authority.

11.2. Risk assessment scheme for metabolites

When a need for the metabolite risk assessment is triggered, as described above in section 11.1, an assessment in line with the EFSA Bee guidance Chapter 11 will be followed to cover the assessment of metabolites.

With regards to toxicity studies, the information requirements on metabolites are in principle the same as for the parent active substance. However, studies on acute contact risk to bees are not considered relevant as exposure to metabolites in nectar and pollen via contact is negligible. Furthermore, the EFSA Bee guidance provides different scenarios based on the availability of metabolite data in a dossier. Three different options for the hazard assessment are described in situations where A) the dossier is complete, B) dossier is partially complete and C) data in dossier is missing (screening tier). If no bee toxicity data are available on the metabolite, the EFSA Bee guidance provides a possibility to estimate the toxicity based on the results of toxicity studies conducted with the metabolite for other invertebrate species, or by estimating the toxicity of a metabolite with non-testing methods like (Q)SAR, or presence of the toxophore.

For the exposure estimation, the acute and chronic dietary exposure is considered for the adult bees and chronic exposure for the larvae in the metabolite assessment. In the rare case when data on the measured residues are available, these can be used in the exposure characterisation. In other cases, the dietary models as for the parent active substance will be applied in the metabolite exposure assessment, however, by taking into account the metabolite formation fraction. The exposure assessment may be started with a screening step and proceeded to Tier 1 if necessary, allowing a stepwise approach for the risk assessment.

Higher tier studies (field effect studies) may eventually be conducted to address the suspected risk from the metabolites if the screening level and lower tier assessment results in unacceptable risks to bees (Chapter 10).

12. Mixtures

In this chapter, an approach is presented on how to address the risk of biocidal products containing more than one active substance for bees (mixtures). The basic concept of the risk assessment for bees is that they are exposed to residues of active substances in the environment, e.g., via their diet. This approach differs in two ways from the standard risk assessment for mixtures for aquatic and terrestrial organisms as presented in the BPR Guidance Vol. IV, Parts B+C (2017):

- The risk assessment for biocidal products for bees in this guidance is a simplified version of the approach for PPPs presented in Chapter 12 of the EFSA Bee guidance. It is the logical consequence of following the approach of the risk assessment for single active substances as presented in this guidance (see Chapters 5 to 7), which is also based on the concept presented in the EFSA Bee guidance.

- Whereas for aquatic and terrestrial organisms according to the BPR Guidance Vol. IV, Parts B+C (2017), where the assessment for mixture toxicity is performed for the most sensitive trophic level, in this guidance the assessment for mixture toxicity for bees is required only for honey bees (and only qualitatively for bumble bees and solitary bees as long as no defined protection goal is available for these two bee groups).

12.1. Legal requirements

Regarding the conditions for granting an authorisation for a biocidal product, the Biocidal Product Regulation (BPR, (EU) 528/2012) states in Article 19(2) that "the evaluation of whether a biocidal product fulfils the criteria set out in point (b) of paragraph 1 shall take into account the following factors: […] (d) cumulative effects, (e) synergistic effects." Cumulative and synergistic effects need to be addressed because biocidal products are usually multi-component mixtures of one or more active substances and a range of co-formulants that serve different purposes, e.g., as preservatives, anti-foaming agents, stabilizers, pigments, emulsifiers, solvents, or diluents.

The approach presented in the EFSA Bee guidance builds on existing methods and scientific experience in assessing chemical mixtures. For the sake for harmonisation, the same approach, but simplified, is presented here and applied for biocidal products. Usually, mixture effects are the sum of the individual effects of the active substances at a certain dose (also known as concentration/dose addition). However, sometimes, interactions of mixture components can cause either significantly increased (synergistic) or decreased (antagonistic) effects compared with the effects predicted by concentration/dose addition. Especially interactions that increase the toxicity of a mixture need to be checked carefully. Although synergism occurs rarely, there are already known combination of active substances that result in synergistic effects, especially if known synergists, like piperonyl butoxide (PBO), are included in biocidal products. In a recent study, synergistic effects on honey bees were observed for mixtures containing thiamethoxam in combination with cyfluthrin and permethrin, respectively (Wenhong et al., 2023).

Both regulations (BPR and PPPR) base the mixture toxicity risk assessment on two options that are considered most adequate for the assessment of hazards and risks of mixtures: measured ("whole mixture" approach) and calculated mixture toxicity ("component based" approach). Generally, calculated mixture toxicity is the preferred option since no additional testing is required. This approach is usually done for the standard aquatic and terrestrial risk assessment for biocidal products. However, for the present bee risk assessment, effect studies with biocidal products that contain two or more active substances are always required (see Table 42 in Chapter 6.1.3). Therefore, in most cases, the risk will be estimated using measured mixture toxicity unless experimental testing of the product is technically not feasible. In the latter case, the calculated mixture toxicity approach would be applied. Based on the mixture toxicity (measured or calculated) selected for each risk case (i.e., acute-contact, acute-dietary, chronic-dietary and larvae-dietary), a combined risk assessment can be conducted for each bee group, in line with the approach presented in Chapter 7.

12.2. Risk assessment for mixtures

12.2.1. Defining the effects

For the **measured mixture toxicity**, the selection of the relevant effect parameters ($DRC_{j,mix\text{-}meas}$) will follow the same rules as explained in Chapter 6. It is important that the selection of dose-response model and the corresponding effect parameters ($DRC_{j,mix\text{-}meas}$) would ensure that the mortality is not underestimated at the lower doses by choosing a model with a too steep slope (see also Section 6.3).

If no effect data of the biocidal product are available, the effect parameters for the **calculated mixture toxicity** ($DRC_{j,mix\text{-}calc}$) need to be estimated. With a dose addition approach, for a mixture of n components, a specific $LD_{x,mix\text{-}calc}$ resulting in an effect level x is calculated as follows:

$$LDx_{mix-calc} = \left(\sum_{i=1}^{n} \frac{p_i}{LDx_i} \right)^{-1}$$ **Equation 37**

Where:
n: number of mixture components
i: index from 1…n mixture components
p_i: the i^{th} component as a relative fraction of the mixture composition (note: Σp_i must be 1)
$LD_{x,i}$: dose of component i provoking x% effect

This means that, when the dose-response relationships and thereby different effect levels ($LD_{x,i}$ from 1% to 99%) and the relative fractions (p_i) of the *n* components of the mixtures are known, it is possible to calculate the $LD_{x,mix-calc}$ for a range of effect levels. This allows describing the dose-response curve of the mixture in a rather precise way, by using the most suitable model to extract the effect parameters ($DRC_{j,mix-calc}$).

Even if the calculated $LD_{x,mix-calc}$ for a range of effect levels cannot be approximated by a log-logistic model or any other model (see Chapter 6), and none of the models results in a $DRC_{j,mix-calc}$ with a good fit, an estimation of the $LD_{x,mix-calc}$ of the mixture due to a specific level of exposure can still be made, by predicting with reasonable accuracy, the effects caused by a certain exposure level by using Equation 37 only.

12.2.2. Defining the exposure

The calculated or measured $LD_{50,mix}$ of a mixture with active substances can be conceived as an LD50 of a single virtual compound. Therefore, in analogy, it can also be assumed for the exposure side that that mixture components together constitute a virtual compound and thus the individual PEQj of each active substance can be added up. This concept is a standard procedure for the standard risk assessment for aquatic and terrestrial risk assessment for biocidal products (BPR Guidance Vol. IV, Parts B+C).

The dietary and contact exposure level of the mixture ($PEQ_{j,mix}$) can be calculated with Equation 38. With this equation, it is assumed that the PEQ_i of all active substances present in the biocidal product will occur at the same moment and are not separated in time (i.e., worst-case $PEQ_{j,mix}$).

$$\boldsymbol{PEQ_{j,mix} = \sum_{i}^{n} PEQ_{j.i}}$$ **Equation 38**

With:

PEQ_i = Predicted Exposure Quantity of active substance i for risk case j. This is the output of the exposure estimation (see Chapter 5).

PEQ_{mix} = Sum of the individual PEQ_i

*It should be carefully checked whether **metabolites** of ecotoxicological relevance have to be included into the PEQ_{mix} or not* (see Chapter 11). Usually, metabolites of ecotoxicological relevance need to be included in the risk assessment of biocides, and therefore also in the mixture toxicity assessment. However, as described in Chapter 11, exposure and effect data are not straightforward to obtain for biocidal uses. In case the applicant pursues to perform studies on metabolites, it should be carefully checked that the gathered information is also useful for the risk assessment of mixtures. For this first version of the ECHA Bee guidance, however, in absence of specific study designs for metabolites, the focus of the mixture toxicity remains on the active substances and thus metabolites shall not be considered for the time being. However, if reliable data on both exposure and effect can be collected, the approach how to conduct the

mixture toxicity risk assessment including metabolite is described in Chapter 12 of the EFSA Bee guidance.

If on this basis the risk is not excluded, no further refinement is possible because in contrast to PPPs, more detailed consideration of time-dependent exposure patterns (i.e., shift in the composition in the environment) is not foreseen for the exposure side (see Chapter 5).

*So far uncertainty remains on the real fate of other **co-formulants** present in the mixture that is applied. Co-formulants may in some cases dissipate slower than the active substances and are not covered at the screening level and Tier 1 risk assessment. In absence of specific data, uncertainty remains on the actual exposures of and effects in bees to these compounds.*

Thus, only if exposure and effect data of an ecotoxicologically relevant co-formulant is available, the concerned co-formulant could be included in the risk assessment of the mixture (see step 2 in Figure 18).

12.2.3. Risk assessment scheme

A detailed step-wise decision scheme is presented below (Figure 18). The scheme needs to be iterated for each risk case.

The steps are identical to the scheme presented in Chapter 12 of the EFSA Bee guidance, apart from the simplification based on omission of step 3 presented in Figure 22 in the EFSA Bee guidance. This step 3 is relevant for PPPs when the exposure estimations were refined based on substance-specific parameters that result in re-calculating the SV parameters with a Monte Carlo method (see Section 5.5.7 of the EFSA Bee guidance). Since for the exposure estimation of biocidal products no such re-calculation of SV parameters is foreseen (only a change in a single value parameter depending on the source of exposure, see Chapter 5), this step can therefore be omitted.

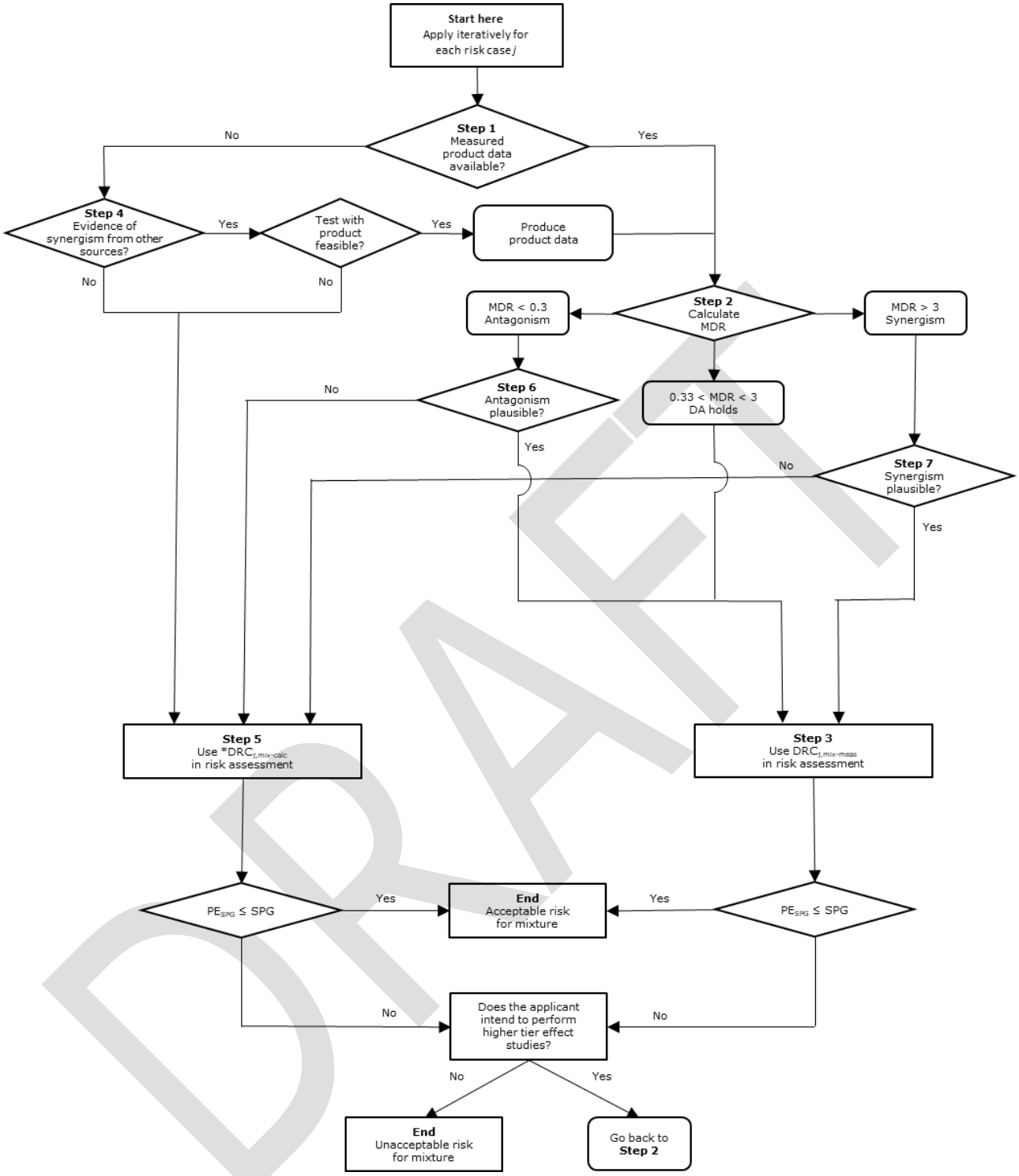


Figure 18: Workflow illustrating the risk assessment scheme for mixtures (modified from EFSA Bee guidance). $DRC_{j,mix-meas}$ = measured dose-response curve for risk case j, $DRC_{j,mix-calc}$ = calculated dose-response curve for risk case j, MDR = model deviation ration; PE = overall predicted effect at colony level; SPG = specific protection goal. *$DRC_{j,mix-calc}$ may need to be corrected by an appropriate MDR from other risk cases/species when synergism is plausible (see step 4).

Step 1. *Are measured mixture toxicity data ($LD50_{j,mix-meas}$) with the product available for the given risk case?*

No, (only data for the a.s. ($LD50_{j,a.s.}$) are available): Go to step 4

Yes, (both data for mixture ($LD50_{j,mix-meas}$) and active substances ($LD50_{j,a.s.}$) are available: Go to step 2.

Step 2. *Check the plausibility of the calculated mixture toxicity $LD50_{j,mix-calc}$ (derived with equation 37) against the measured mixture toxicity ($LD50_{j,mix-meas}$) on the basis of the mixture composition of the active substances in the product by means of the Model Deviation Ratio (MDR, see equation 39*).

Notes:

In order to determine if the active substance may act more (i.e., synergistically) or less (i.e., antagonistically) than expected by dose addition, a comparison of the calculated $LD50_{j,mix-calc}$ versus the measured $LD50_{j,mix-meas}$ endpoints is informative.

This comparison may also indicate that other co-formulants not included in the calculation of $LD50_{j,mix-calc}$ could contribute to the overall mixture toxicity in an appreciable way. When this is the case, they can be included in a refined calculation, however only if the respective single-compound toxicity data of the co-formulant is available which is very rarely the case. Possible outcome of the MDR calculation is the following:

$$MDR = \frac{LD50_{j,mix-calc}}{LD50_{j,mix-meas}}$$ **Equation 39**

- ***0.33≤MDR≤3.*** *The measured and calculated $LD50_j$ are considered in agreement if the MDR is between 0.33 and 3. That is the dose addition hypothesis holds. This convention is in line with the recent EFSA recommendations related to pesticide RA (Pesticide Peer Review Meeting 185, 9–12 October 2018). In relation to 'when a formulation should be considered more toxic than the active substance', the proposal was to account for a difference of a factor of three, as recommended in the guidance from the Directorate-General for Health and Food Safety (SANCO/10597/2003 rev. 10.1) (European Commission, 2012) on the equivalence of batches. Thus, if the MDR lies between these two values, it is considered that the dose addition hypothesis holds*
- ***MDR is < 0.33.*** *Less than additive (i.e., antagonistic) mixture toxicity is indicated if the MDR is below 0.33.*
- ***MDR is > 3.*** *More than additive (i.e., synergistic) mixture toxicity is indicated if the MDR is > 3.*

A careful interpretation of the MDR is mandatory, especially if not all components that potentially contribute to the measured mixture toxicity (e.g., co-formulants) have been considered in the calculated mixture toxicity. Care should also be taken that the counter-checking of measured and calculated $LD50_j$ refers to the same basis, that is, the relative proportion of mixture components must be consistent (e.g., to the sum of active substances of a given biocidal product if co-formulants are not included in the dose addition calculation).

If MDR = 0.33–3 (dose addition approximately holds for the mixture): Go to step 3 (use measured mixture toxicity)
If MDR < 0.33 (mixture less toxic than dose addition): Go to step 6
If MDR > 3 (mixture more toxic than dose addition): Go to step 7

Step 3 (Step 4 in EFSA Bee guidance). *Use the measured mixture dose-response ($DRC_{j,mix-meas}$) and proceed to the risk assessment as described in Chapter 7.*

PE_{SPG} ≤ SPG: Acceptable risk
PE_{SPG} > SPG: Acceptable risk not demonstrated

Step 4 (Step 5 in EFSA Bee guidance). *Is there evidence that synergistic interactions between*

mixture components might occur[15] which cannot be ruled out for the given endpoint with sufficient certainty?

Note: If synergistic effects cannot be excluded, the risk assessment should preferably be based on measurements, as synergistic interactions are not predictable by dose addition nor by other concepts such as independent action/response addition alone. If experimental testing of the mixture is no option (e.g., for technical reasons) for certain species and endpoints, but synergism is known form other studies, the risk assessment may be performed by ***shifting the calculated*** $DRC_{j,mix\text{-}calc}$ ***by the MDR*** *obtained from other risk cases/species if available.*

Yes (mixture toxicity calculation not feasible): Measured mixture toxicity data required for risk assessment

- *If measured mixture toxicity becomes available: Go to step 2*
- *If measuring the mixture toxicity is not technically feasible, but a reliable MDR is available from other risk cases/species, shift the calculated* $DRC_{j,mix\text{-}calc}$ *by the MDR and go to step 5*

No (mixture toxicity calculation feasible): Go to step 5 (use calculated mixture toxicity)

Step 5 (Step 6 in EFSA Bee guidance). *Use the calculated* $DRC_{j,mix\text{-}calc}$ *to estimate the effect for the risk case of concern and proceed to the risk assessment.*

$PE_{SPG} \leq$ SPG: Acceptable risk
$PE_{SPG} >$ SPG: Acceptable risk not demonstrated, check single-substance refinement options

Note that PE_{SPG} *represents the combined effects of all the risk cases taken in consideration in the RA. To comply with the SPG, any risk case can be refined independently of the others and thus a refinement on another risk case may suffice.*

Step 6 (Step 7 in EFSA Bee guidance). *Carefully recheck the apparent antagonism as observed in the measured mixture toxicity data (*$DRC_{j,mix\text{-}meas}$*) regarding potential impacts of the default assumption of dose addition (e.g., check for heterogenous input data, i.e. different study designs/endpoints). Does the apparent antagonism hold?*

Note: If plausible toxicological explanation for this apparent antagonism can be provided (e.g., special feature of the formulation type), the risk assessment should be based on the measured mixture toxicity. Otherwise, the calculated mixture toxicity is a better option. No correction for MDR is needed, as the calculated mixture toxicity represents a worst-case.

Yes (antagonism holds): Go to step 3 (use measured mixture toxicity)
No (antagonism does not hold): Go to step 5 (use calculated mixture toxicity)

Step 7 (Step 8 in EFSA Bee guidance). *Carefully recheck the apparent synergism as observed in the measured mixture toxicity data (*$DRC_{j,mix\text{-}meas}$*) regarding potential impacts of heterogeneous input data (testing conditions/endpoints should be homogenous) and of co-formulants ignored in the dose addition calculation. Does the apparent synergism hold?*

Note: If plausible toxicological explanation for this apparent synergism is available or if this check reveals the presence of a toxic co-formulant, the risk assessment should be based on the

[15] e.g., based on toxicological knowledge from literature, some indications are given in Appendix 11 in the BPR Vol. IV Parts B+C, ECHA 2017

measured mixture toxicity. Otherwise, the calculated mixture toxicity is a better option.

Yes (synergism holds): Go to step 3 (use measured mixture toxicity)
No (synergism does not hold): Go to step 5 (use calculated mixture toxicity)

13. Risk mitigation measures, instructions for use, and warning sentence

13.1. Risk mitigation measures

If an unacceptable risk to bees is indicated, consideration of risk mitigation measures (RMMs) is one possible option to reduce the exposure of bees and to refine the risk assessment. RMMs can be integrated to an exposure assessment re-estimation at any tier, except the screening level and/or they can be proposed to reformulate the problem formulation. Any suggested mitigation has to reduce the risk sufficiently so that the risk assessment indicates an acceptable risk. Furthermore, risk mitigation measures should be practicable (e.g., for the proclaimed user category), suitable for the intended use, and enforceable. It has to be assured that risk mitigation phrases are seen by a relevant person (product user).

RMMs can be classified into two broad categories with the aim to reduce the exposure to bees:

1. Specific mitigation measures (quantifiable) are targeted actions which are needed to mitigate an identified risk due to biocide exposure. The mitigation must be demonstrated quantitatively. Any suggested mitigation must be accompanied by an appropriate risk assessment for which additional data may be needed. Specific mitigation measures can be proposed by the applicant within the risk assessment process.

2. Generic mitigation measures (unquantifiable) are those actions which are undertaken to manage the risk to bees. Generic mitigation measures can be considered as risk management options within the decision-making process for the approval of active substances or the authorization of biocidal products.

It is important that the effect of implementing risk mitigation measures on other sections is taken into account, especially the effect on efficacy.

Specific risk mitigation measures can include for example the reduction of the application rate or the number of applications and/or increase of interval between applications.

Currently the only (generic) harmonized risk mitigation phrase aimed at reducing the exposure and hence the risk to bees is the following SPe8 from Annex V of Directive 1999/45/EC[16]:

> *Dangerous to bees./To protect bees and other pollinating insects do not apply on flowering crops./Do not use where bees are actively foraging./Remove or cover beehives during application and for (state time) after treatment./Do not apply when flowering weeds are present./Remove weeds before flowering./Do not apply before (state time).*

This phrase, or parts of it, is considered to cover risk mitigation for PPPs for bees, specifically honey bees. The sentences may be considered for applications of biocides that are comparable

[16] COMMISSION DIRECTIVE 2003/82/EC of 11 September 2003 amending Council Directive 91/414/EEC as regards standard phrases for special risks and safety precautions for plant-protection products

to those of PPPs, specifically for large-scale spray applications (see Sections 5.6 to 5.7) and potentially for small-scale spraying on walls and foundation of houses (see Section 5.3). It must be emphasized that the sentences would need to be adapted for biocide use. For example, for biocides there is usually no distinct differentiation between "crop" and "field margin". Furthermore, as most biocides are applied on or in the proximity of mixed vegetation, it might not be possible to avoid flowering plants/weeds during application.

Furthermore, parts of this phrase are associated with uncertainty regarding the practicability, and potentially have unclear or even undesired effects.

For the biocides source of exposure due to manure/sewage sludge application (see Section 5.2), there are no appropriate RMMs to reduce the consumption of contaminated pollen and nectar by bees, other than the measures to reduce the amount of biocide reaching manure/sludge. Manure as an agricultural asset is exported/traded and therefore restrictions, such as limiting the amount of manure that is applied or the type of land that it is applied to, cannot be expected to be complied with via supply chain. Exposure of bees can only be limited indirectly by measures limiting the amount of biocide reaching manure. RMMs or instructions for use that are generally aimed at reducing environmental input (i.e., by preventing the product from reaching the manure), and therefore only having an indirect effect on bee exposure, are not considered in this guidance. These can be applied/used in accordance with other available guidance and current practice.

13.2. Instructions for use for baits

RMMs are applied when an unacceptable risk is identified based on the conclusions of the risk assessment. For bait application, no quantitative risk assessment is proposed in this guidance. Baits are generally not expected to be attractive to bees for several reasons (see Section 2.1.2). Indirect exposure of bees through soil is not considered relevant for baits because the area of soil contaminated from leaching or run-off is very local and small-scale (See Section 2.1.2).

One key factor, that determines whether bees can orally take up bait formulations, is the viscosity. It is known that viscous sugar-based substrates need to be liquefied before bees would be able to take it up. To prevent baits that are placed outdoors from being liquified, it is recommended to include the following instruction for use on the label of the bait product:

Apply only in areas that are not liable to submersion or becoming wet, i.e., protected from rain, floods, and cleaning water.

This instruction for use is already commonly used for bait products with outdoor application because it prevents the formulation from entering the environment. It is applicable to most bait formulations, i.e., bait boxes, granules, and gels.

Several RMMs/instructions for use for baits have been proposed and discussed by the Environment Working Group of the Biocidal Products Committee, and by the representatives of Member State competent authorities (MSCAs) and the European Commission. The discussed measures/sentences were aimed at preventing direct consumption of baits by bees. In the discussion by the biocide competent authorities, it was noted that the proposed sentences might be disproportionate considering there is no risk identified due to the lack of appropriate risk assessment tools[17]. Furthermore, it was proposed that the sentences are considered in the context of the ECHA Bee guidance development.

[17] CA-Dec20-Doc.4.1 WARNING SENTENCE AND RMM FOR BEES. Available at https://circabc.europa.eu/ui/group/e947a950-8032-4df9-a3f0-f61eefd3d81b/library/5e6cf719-8286-4cbf-9b1e-f01eade08bb7/details

During guidance development, the ECHA EG conducted a survey among stakeholders and experts to assess whether these and similar sentences are considered efficient in preventing bees and other pollinators from accessing the baits. The responses to the survey generally lacked data to support the statements (e.g., literature, laboratory, or field studies) and therefore only limited recommendations are made in the current ECHA Bee guidance.

The provided answers generally suggested that *bait boxes* with small enough openings are considered to prevent bees from accessing the bait inside the bait box. Bait boxes can therefore be considered as safe for bees. However, applying a product in a bait box without further efficacy testing is not an option in most cases.

Misuse in general and specifically of bait products is not covered in the ECHA guidance. Users are generally expected to follow instructions for use. However, several incidents with biocides with fatal bee hive intoxication were reported within the last years in Switzerland for example. In few cases, baits were directly used in bee hives to combat ants. To raise awareness and prevent such incidents, the following sentence may be added to the instructions for use:

Do not apply in or near bee hives.

13.3. Hazard-based Warning Sentence

Prior to the development of the ECHA Bee guidance, the procedure to include a hazard-based warning sentence in the authorisation of biocidal products was discussed by the Member States competent authorities[17]. The following wording was agreed:

"*This biocidal product contains (active substance name) which is dangerous to bees*".

Further on, the biocide Environment Working Group concluded that a warning sentence shall be applied for all biocidal products used outdoor under PT18, PT19 and PT08 containing an active substance used as an insecticide, acaricide or product to control other arthropods which is found to be below the toxicity threshold. In the case of PT8 products the warning sentence will only be used for products applied in-situ outdoor and not to treated wood.

With regards to the toxicity threshold, the following was considered:

> An active substance would be found to be below the toxicity threshold if a standard contact or oral acute LD_{50} datapoint on adult honeybees, bumble bees or solitary bees exists for that substance and is below 11 µg/bee (OECD 213 and 214, for instance). In case there are more than one datapoints available, the one showing the lowest LD_{50} should be considered. Information that has been submitted for the same substance for other regulatory frameworks (e.g., PPPR) can also be used. Additionally, literature data on acute endpoints can also be used to compare with the threshold if the studies are reliable and relevant. In the absence of studies performed according to standard guidelines and reliable and relevant literature data demonstrating that the substance is below the toxicity threshold, no scientific evidence exists which could enable an assessment of hazard properties to bees.

This agreed procedure was an interim solution until the ECHA Bee guidance is available.

With development of the ECHA Bee guidance and implementation of the risk assessment to bees, the idea to add a hazard-based warning sentence to product labels is still valid. Even if a risk assessment for bees for a certain product shows an acceptable risk, the intrinsic toxic properties of the active substance to bees are a reason to add a warning sentence on the product label. This would raise awareness of users of the (intrinsic) toxic properties of the active substance in the product and may lead to a more careful and correct (according to the use instructions) use of the product. Furthermore, a warning sentence could steer users to buy products which do not

have the warning sentence, because they contain active substances not toxic for bees or are used in a way where the product is not accessible for bees (e.g., gels in bait boxes). Finally, the current guidance document does not propose a risk assessment for non-bee pollinators due to a lack of data. The warning sentence could therefore also protect NBPs from the intrinsic toxic properties of the active substance.

The ECHA Bee guidance suggests that the following **scientific criteria** should be used to decide, to which PT 18 products the warning sentence shall be applied: toxicity of active substance(s) below the threshold of LD_{50} (acute oral or acute contact) < 11µg/bee (HB, BB or SB).

The warning sentence shall be applied regardless of the concentration of the active substance in the product. Products with the following area or use/use pattern shall have the warning sentence:

- products used outdoors
- products used in animal housings (as there are open or half-open animal housings and many farmers also keep bees in close proximity to stables).

There are two **exceptions**, when it is not necessary to apply the warning sentence for the product, although the toxicity is below the threshold:

- products exclusively used indoors in households
- products used outdoors, but in bait boxes

In both cases it is not expected that bees are exposed to the product.

14. Conclusions

The ECHA Bee guidance document provides applicants and competent authorities with the methodology to assess the risk to honey bees, bumble bees and solitary bees from the use of biocidal products. The guidance takes into account the available guidance for plant protection products (EFSA 2023), having made the necessary adaptations to biocides when needed.

With regards to arthropod pollinators other than bees, future development of guidance is needed since at the time of the preparation of this guidance sufficient information was not available for developing a risk assessment methodology for non-bee pollinators.

15. Recommendations

For general recommendations, please refer to the Chapter 15 of the EFSA Bee guidance.

[*Note to reader: The ECHA EG will consider the biocide-specific recommendations after the written consultation has finished, taking into account comments received and related further discussions. The below sub-chapters are placeholders to be completed after consultation process*.]

15.1. Inclusion of potentially important matrices/exposure routes/life stages

15.2. Exposure Assessment

15.3. Effect assessment in lower tiers

15.3.1. Lower tier risk assessment

15.3.2. Sublethal effects on honey bees in risk assessment

15.3.3. Higher tier risk assessment

15.3.4. Metabolites

15.3.5. Mixtures

16. References

Biocidal Product Regulation (528/2012/EC, BPR). (2012). Retrieved from https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex:32012R0528

Bliss, C. I. (1939). The toxicity of poisons applied jointly. *Annals of Applied Biology, 26*(3), pp. 585-615. doi:https://doi.org/10.1111/j.1744-7348.1939.tb06990.x

ECHA (European Chemicals Agency). (2017). Guidance on the BPR: Volume IV Environment, Assessment & Evaluation (Parts B+C).

ECHA (European Chemicals Agency). (2020, December). Preliminary considerations for ECHA's guidance on the "Methodology to assess the risk to bees and other non-target arthropod pollinators from the use of biocides". Retrieved from https://echa.europa.eu/documents/10162/17234/scoping_paper_pollinators_guidance_en.pdf/7957c0f8-5ded-4a6e-17a7-2a899bbb141a?t=1608116526557

ECHA (European Chemicals Agency). (2022, October 14). Technical Agreements for Biocides (TAB) - ENV. Helsinki, Finland. Retrieved from file://echa/data/users/u23060/Roaming%20Profile/Downloads/ENV-TAB_DB_2022_10_14.pdf

ECHA (European Chemicals Agency). (n.d.). Guidance on the Biocidal Products Regulations.

ECHA (European Chemicals Agency); Hätönen, M.; Kantner, C.; Losada, L. i L. R.; Ludwig, N.; Gonzalez, A. B.; Riedhammer, C.; Kunz, P.; Panico, S. C.; Laakkonen, E.; Dolcet, L. P.; Vangheel, M.; Carlon, C.; Alonso, S. G. (2022, September). European arthropods and their role in pollination: scientific report of their biodiversity, ecology and sensitivity to biocides. Helsinki, Finland. doi:10.2823/147199

EFSA (European Food Safety Authority). (2010). Scientific Opinion on the development of specific protection goal options for environmental risk assessment of pesticides, in particular in relation to the revision of the Guidance Documents on Aquatic and Terrestrial Ecotoxicology (SANCO/3268/2001 and SA. Retrieved from https://www.efsa.europa.eu/en/efsajournal/pub/1821

EFSA (European Food Safety Authority). (2016). Guidance to develop specific protection goals options for environmental risk assessment at EFSA, in relation to biodiversity and ecosystem services. Retrieved from https://www.efsa.europa.eu/en/efsajournal/pub/4499

EFSA (European Food Safety Authority). (n.d.). Revised guidance on the risk assessment of plant protection products on bees (Apis mellifera, Bombus spp. and solitary bees).

EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues) (PPR). (2012). Scientific Opinion on the science behind the development of a risk assessment of Plant Protection Products on bees (Apis mellifera, Bombus spp. and solitary bees). *Efsa Journal, 10*(5), 2668. doi: https://doi.org/10.2903/j.efsa.2012.2668

EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues). (2010). Scientific Opinion on the development of specific protection goal options for environmental risk assessment of pesticides, in particular in relation to the revision of the Guidance Documents on Aquatic and Terrestrial Ecotoxicology. *EFSA Journal, 8*(10), 1821. doi:10.2903/j.efsa.2010.1821

EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues). (2013). Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. *EFSA Journal, 11*(7), 3290. doi:https://doi.org/10.2903/j.efsa.2013.3290

EFSA Scientific Committee. (2016). Guidance to develop specific protection goals options for environmental risk assessment at EFSA, in relation to biodiversity and ecosystem services. *EFSA journal, 14*(6), 4499. doi:https://doi.org/10.2903/j.efsa.2016.4499

EFSA Scientific Committee, More SJ, Bampidis V, Benford D, Bragard C, Halldorsson TI, Hernández-Jerez AF, Bennekou SH, Koutsoumanis K, Lambré C, Machera K, Mennes W, Mullins E, Nielsen SS, Schrenk D, Turck D, Younes M, Aerts M, Edler L, Sand S, Wright M, Binaglia M, Bottex B, Abrahantes JC and Schlatter J. (2022, October). Guidance on the use of the benchmark dose approach in risk assessment. *Efsa Journal, 20*(10), e07584. doi:https://doi.org/10.2903/j.efsa.2022.7584

EFSA Working Group. (2022). Revised guidance on the risk assessment of plant protection products on bees (Apis mellifera, Bombus spp. and solitary bees). *EFSA Journal*. doi: 10.2903/j.efsa.201Y.xxxx10.2903/j.efsa.201Y.xxxx

European Commission. (2003, june). Guidance Document for Environmental Risk Assessments of Active Substances used on Rice in the EU for Annex I Inclusion. Document prepared by Working Group on MED-Rice, EU Document Reference SANCO/1090/2000-rev. 1. Brussels, Belgium. Retrieved from https://1library.net/document/q7wpdvrd-guidance-document-environmental-sessments-active-substances-annex-inclusion.html

European Commission. (2020, December). Doc.4.1 Warning sentence and RMM for bees_final, rev 1 96th CA-June-2022, rev 2 97th CA October 2022. Retrieved from https://circabc.europa.eu/ui/group/e947a950-8032-4df9-a3f0-f61eefd3d81b/library/5e6cf719-8286-4cbf-9b1e-f01eade08bb7/details

FOCUS (FOrum for the Co-ordination of pesticide fate models and their USe). (2001). FOCUS Surface Water Scenarios in the EU Evaluation Process under 91/414/EEC". Report of the FOCUS Working Group on Surface Water Scenarios, EC Document Reference SANCO/4802/2001-rev.2. 245 pp. Retrieved from https://esdac.jrc.ec.europa.eu/projects/surface-water

Green, J. W., Springer, T. A., & Holbech, H. (2018). *Statistical Analysis of Ecotoxicity Studies.* John Wiley & Sons. doi:10.1002/9781119488798

Kim, W., Gilet, T., & Bush, J. W. (2011). Optimal concentrations in nectar feeding. *Proceedings of the National Academy of Sciences, 108*(40), 16618-16621. doi:https://doi.org/10.1073/pnas.1108642108

Li, W., Lv, L., Wang, Y., & Zhu, Y.-C. (2023). Mixture effects of thiamethoxam and seven pesticides with different modes of action on honey bees (Aplis mellifera). *Scientific Reports, 13*(2679). doi:https://doi.org/10.1038/s41598-023-29837-w

Mangan, R., & Tarshis Moreno, A. (2009). Honey Bee Foraging Preferences, Effects of Sugars, and Fruit Fly Toxic Bait Components. *Journal of Economic Entomology, 102*(4), 1472-1481. doi:https://doi.org/10.1603/029.102.0411

Nicolson, S., de Veer, L., Köhler, A., & C.C.W., P. (2013). Honeybees prefer warmer nectar and less viscous nectar, regardless of sugar concentration. *Proceedings of the Royal Society B, 280*(1767). doi:https://doi.org/10.1098/rspb.2013.1597

OECD (Organisation for Economic Co-operation and Development). (1998). Test No. 213: Honeybees, Acute Oral Toxicity Test, OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris. doi:https://doi.org/10.1787/9789264070165-en

OECD (Organisation for Economic Co-operation and Development). (1998). Test No. 214: Honeybees, Acute Contact Toxicity Test, OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris. doi:https://doi.org/10.1787/9789264070189-en

OECD (Organisation for Economic Co-operation and Development). (2006). OECD Environmental Health and safety Publications. Series on Emission Scenario Documents No. 14. Paris. Retrieved from https://echa.europa.eu/documents/10162/983773/pt18_insecticides_for_stables_and_manure_en.pdf/cc437f66-35ef-4116-a281-026c4f2fb6d0?t=1377104815277

OECD (Organisation for Economic Co-operation and Development). (2006). Test No. 54: Current Approaches in the Statistical Analysis of Ecotoxicity Data: A Guidance to Application. Paris. doi:https://doi.org/10.1787/9789264085275-en

OECD (Organisation for Economic Co-operation and Development). (2007). Test No. 501: Metabolism in Crops, OECD Guidelines for the Testing of Chemicals, Section 5. Paris, France. doi: https://doi.org/10.1787/9789264061835-en.

OECD (Organisation for Economic Co-operation and Development). (2008). Environmental Health and safety Publications. Series on Emission Scenario Documents No. 18. Paris. Retrieved from https://echa.europa.eu/documents/10162/983773/pt18_oecd_esd_household_professional_uses_en.pdf/bb353df7-5606-41d2-b799-ea15717a8d4b?t=1377104820815

OECD (Organisation for Economic Co-operation and Development). (2016). Test No. 239: Guidance Document on Honey Bee (Apis mellifera) Larval Toxicity Test, Repeated Exposure. Paris. Retrieved from https://one.oecd.org/document/env/jm/mono(2016)34/en/pdf

OECD (Organisation for Economic Co-operation and Development). (2017). Test No. 245: Honey Bee (Apis Mellifera L.), Chronic Oral Toxicity Test (10-Day Feeding), OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris. doi:https://doi.org/10.1787/9789264284081-en

OECD (Organisation for Economic Co-operation and Development). (2017). Test No. 246: Bumblebee, Acute Contact Toxicity Test, OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris. doi:https://doi.org/10.1787/9789264284104-en

OECD (Organisation for Economic Co-operation and Development). (2017). Test No. 247: Bumblebee, Acute Oral Toxicity Test, OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris. doi:https://doi.org/10.1787/9789264284128-en

OECD (Organisation for Economic Co-operation and Development). (2021). Test No. 332: Guidance Document on honey bee (Apis mellifera L.) homing flight test, using single oral exposure to sublethal doses of test chemical. Series on Testing and Assessment. Paris. Retrieved from https://one.oecd.org/document/ENV/CBC/MONO(2021)7/en/pdf

Rao, J. N., & Scott, A. J. (1992). A Simple Method for the Analysis of Clustered Binary Data. *Biometrics, 48*(2), 577-585. doi:https://doi.org/10.2307/2532311

Roessink, I., Hanewald, N., Schneider, C., Exeler, N., Schnurr, A., Molitor, A. M., . . . Van der Steen, S. (2017). A method for a solitary bee (Osmia sp.) first tier acute contact and oral laboratory test: an update. *Proceedings of the Hazards of pesticides to bees - 13th International symposium of the ICP-PR Bee protection group.* Valencia, Spain. doi:10.5073/jka.2018.462.045

Roessink, I., Hanewald, N., Schneider, C., Quambusch, A., Exeler, N., Cabrera, A. R., . . . Soler, E. (2019). Progress on the Osmia acute oral test - findings of the ICPPR Non-Apis subgroup solitary bee laboratory testing. *Proceedings of the Hazards of pesticides to bees - 14th international symposium of the ICP-PR Bee protection group.* Bern, Switzerland. doi:10.5073/jka.2020.465.017

Siviter, H., Bailes, E. J., Martin, C. D., Oliver, T. R., Koricheva, J., Leadbeater, E., & Brown, M. J. (2021). Agrochemicals interact synergistically to increase bee mortality. *nature, 596*, 389-392. doi:https://doi.org/10.1038/s41586-021-03787-7

Smitley, D., Brown, D., Finneran, R., Elsner, E., Landis, J., Shrewsbury, P., . . . Palmer, C. L. (2019, may 01). Better habitat for bees. Retrieved 04 26, 2021, from Michigan State University MSU Extention Pollinators & pollination: https://www.canr.msu.edu/news/better-habitat-for-bees#trees

Toledo-Hernández, R., Pulido-Enríquez, M., Landeros-Pedro, F., Rodríguez, D., & Sánchez, D. (2021). Acttra SWD Bait Formulation Against Drosophila Suzukii and its Compatibility with Berry Pollination by the Honey Bee, Apis Mellifera. *Journal of Apicultural Science, 65*(2), 279-290. doi: https://doi.org/10.2478/jas-2021-0019

Tosi, S., & Nieh, J. C. (2019). Lethal and sublethal synergistic effects of a new systemic pesticide, flupyradifurone (Sivanto), on honeybees. *Proceedings of the Royal Society B, 286*, 20190433. doi:http://dx.doi.org/10.1098/rspb.2019.0433

17. Glossary and/or abbreviations and/or acronyms

[*Draft list – a complete list will be prepared for the final document*]

Abbreviation/Acronym	Explanation
a.s.	Active Substance
BB	Bumble Bee
BBCH	Growth stage; uniform coding of phenologically similar growth stages of all mono- and dicotyledonous plant species (Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie)
BSF	Body surface factor (dm^2/bee)
CA	Concentration Addition
CMP	Food consumption, e.g. CMPsu: sugar consumption
Colony	A colony consists a number of individuals of the same species that are living in close association with each other.
Colony strength	Colony size, defined here as the number of adults that forms the colony
DRC	Dose-Response Curve (the parameter describing the steepness of the dose-response relationship obtained from standard laboratory tests
DW	Downward spray, it includes all application methods where the spray is directed to the ground
EA	Exposure Assessment
EC_{50}	Concentration required killing half the members of a tested population after a specified test duration
EC_x	Concentration with x % level of effect compared to the control
ECHA	European Chemicals Agency

EDx	Effect dose, e.g. ED50 Effect dose for 50% of the organisms tested
EED	Estimated exposure dose (in higher tier effect studies)
EfAGs	Effect Assessment Goals. The EfAG operationalises the Specific Protection Goals with respect to the effect assessment, e.g., definition of relevant model species, type of toxicity, measured endpoints for the relevant species, extrapolation between species.
EFSA	European Food Safety Authority
EREQ	Ecotoxicologically Relevant Exposure Quantity. Conceptual interface between the effect and exposure tiers. It is based on ecotoxicological considerations and defines the type of exposure quantity that in a mechanistic sense best explains observed effects in an ecotoxicological experiment
EU	European Union
EU COM	European Commission
ExAGs	Exposure Assessment Goals. The ExAG operationalises the Specific Protection Goals with respect to the exposure assessment in the environment, e.g., definition of the environmental exposure, type, duration, matrix, and level of conservativeness of the exposure estimate.
FOCUS	FOrum for Co-ordination of pesticide fate models and their Use
FVI	Flower-Visiting Insect
GD	Guidance Document
Guttation	Appearance of drops of xylem sap on the tips or edges of leaves of some vascular Plants
HB	Honey Bee
Hive	Enclosed, man-made structure in which some honey bee or bumble bee colonies with their nets are kept.

Honey dew	A sugary secretion produced by aphids and other insects
Inflection point	Points of the curve where the curvature changes its sign
LD50	Lethal dose, required to kill half the members of a tested population after a specified test duration
LDD_{50}	Median Lethal Dietary Dose (Chronic dietary experiments)
LF	Landscape factor. This factor describes the proportion of the food intake of a bee colony or population that originates from the treated field: e.g. LFpo landscape factor for pollen
MDR	Model Deviation Ratio
NBP	Non-Bee Pollinator
Nest	A nest is a structure built by the bees to hold eggs, offspring, and the adult form(s) itself. Honey bees, bumble bee and solitary bees can have nest(s).
NOEC	No Observed Effect Concentration
NOED	No Observed Effect Dose
OECD	Organisation for Economic Co-operation and Development
PCUD	Predicted Concentration per Unit Dose
PEC	Predicted Exposure Concentration
Protection goal	The objective of environmental policies, typically defined in law or regulations.
PPP	Plant Protection Product
(Q)SAR	(Quantitative) structure-activity relationship
RMM	Risk Mitigation Measures. Actions which are needed to mitigate/manage a risk to bees due to chemical exposure

RUD	Residue per Unit Dose. The parameter expressing the residue concentration of the pesticide molecule in pollen and in nectar, standardised on an application rate of 1 kg/ha
SB	Solitary Bee
SPG	Specific Protection Goal
SSD	Species Sensitivity Distribution.
SUW	Sideward and Upward spray, it includes all applications where the spray is directed sidewards or upwards (this can be air assisted or without air assistance)
SV	Shortcut value. The 90th percentile of a distribution of residue intake per bee (or larvae) over a colony (or population, for solitary bees).
TEF	Toxicity extrapolation factor. Extrapolation factors from standard species to smaller bumble bees and solitary bees for a generic (substance-independent) relationship between LD50 and bee weights
TRR	Total Radioactive residue
TRT	Time-reinforced toxicity. The potential of the compound under evaluation for showing increased toxic effects due to long-term exposure to low doses, compared to what would be expected based on short-term exposure to higher doses
TWA	Time weighted average

1

2

Appendices

Appendix A - Attractiveness of different shrubs and wild trees

Flowering shrubs can be an excellent food source for bees because they tend to grow larger than herbaceous perennials, and therefore produce a larger number of flowers. Some species bloom all summer.
Flowering trees are critical to providing an ample food source for bees because of their large size and thousands of flowers. A blooming linden or black locust produces so much pollen and nectar that it dwarfs the amount provided by most garden flowers in comparison. Among other trees attractive to bees are red maple, hawthorn, chestnut, willow, etc.
Wind pollinated trees are abundant in temperate forests. **Wind-pollinated trees do not produce nectar, but bees may take advantage of them as an abundant source of pollen.** Male flowers cast pollen into the wind in random search of a mate. In early spring, it is not uncommon to see bees and other insects visiting the male flowers in search of pollen, but they are foragers, not pollinators. Among the most frequently visited wind-pollinated trees are ash, birch, elm, hickory, oak, poplar, maple and willow. Pollen from the wind-pollinated trees may be collected by bees because of a favourable nutritional value, the large amount of pollen produced, or because it is available at times when other food sources are scarce. Oaks are self-incompatible, incapable of pollinating themselves to produce viable acorns. The bees are not pollinators unless they carry their collected pollen to female flowers on another tree. **Oak trees should be therefore considered as attractive to bees for pollen only.** Also, several genera of wind-pollinated angiosperms are routinely visited by bees to collect pollen (Smitley et al).
Pines, spruces and nearly all gymnosperms are not usually visited by bees unless it is to gather sap used for propolis, a sticky substance used to fill crevices and seal hives. Such trees **should be considered as non-attractive to bees for pollen nor nectar**.[18],[19]

Appendix B – Shortcut values for contact and dietary exposure

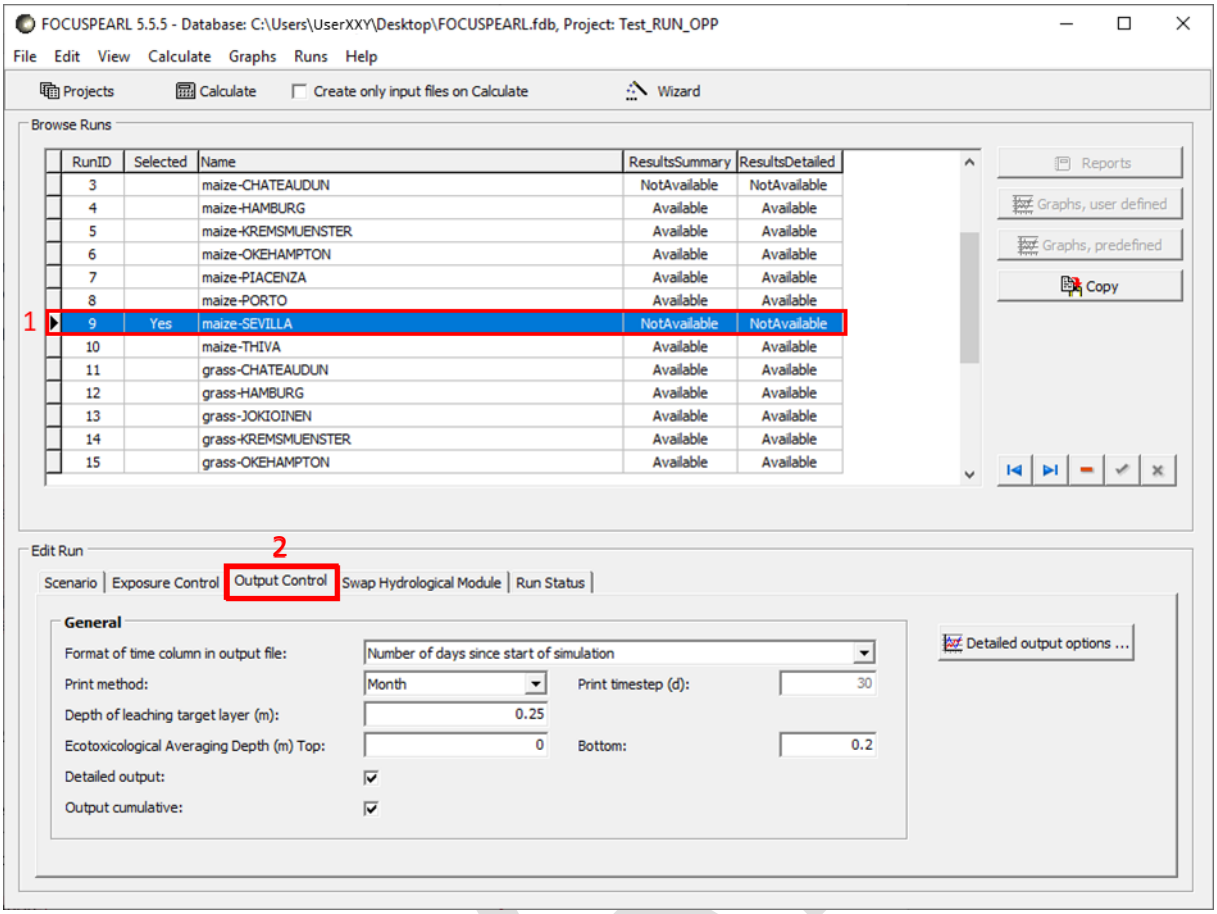
See separate document *ECHA_Bee_guidance_Appendix_B_DRAFT*.

Appendix C – Manual for Refinement of PECpw with FOCUS PEARL

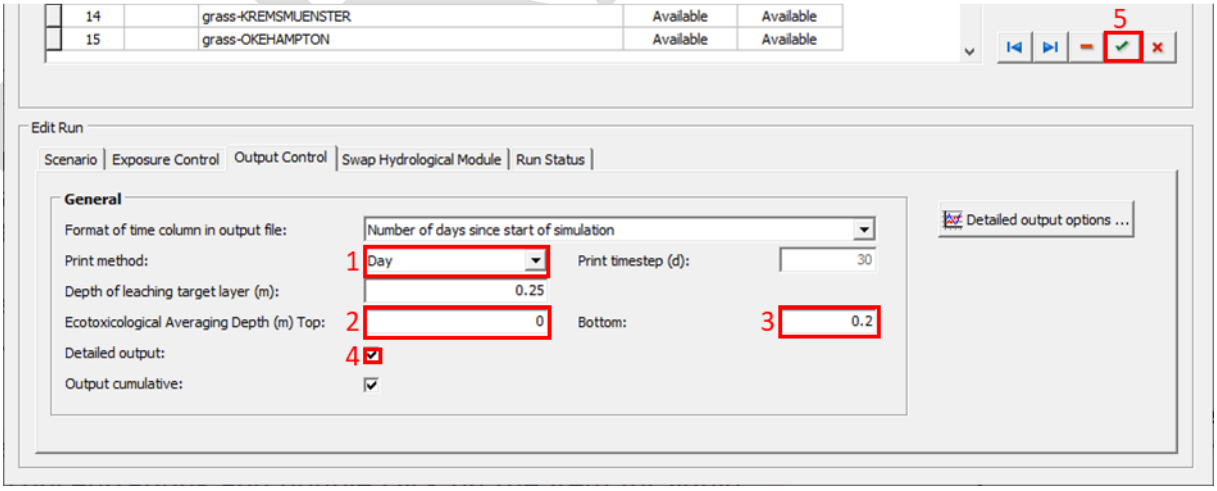
So far, FOCUS PEARL has only been used for groundwater modeling in the Biocides assessment. A refinement of PEC_{pw} (in alignment with Tier3-A of EFSA Guidance) is possible with FOCUS PEARL 5.5.5.
A detailed approach to obtain output on the pore water concentration in FOCUS PEARL 5.5.5 is given below. The starting point for creating an output on pore water concentration are the "runs" produced when modelling groundwater concentrations. For this, the basic settings on the substance, application and scenario selection (which crops) are required (please also consider TAB ENV 23, 165 and 166 (2022)):

- 1 Select the run of interest, go to the Output Control tab.
  This approach should be followed for all locations and application schemes (arable land/grassland). Here in the example, it was made for RunID 9 (Sevilla arable land with maize). Then, go to the Output Control tab.

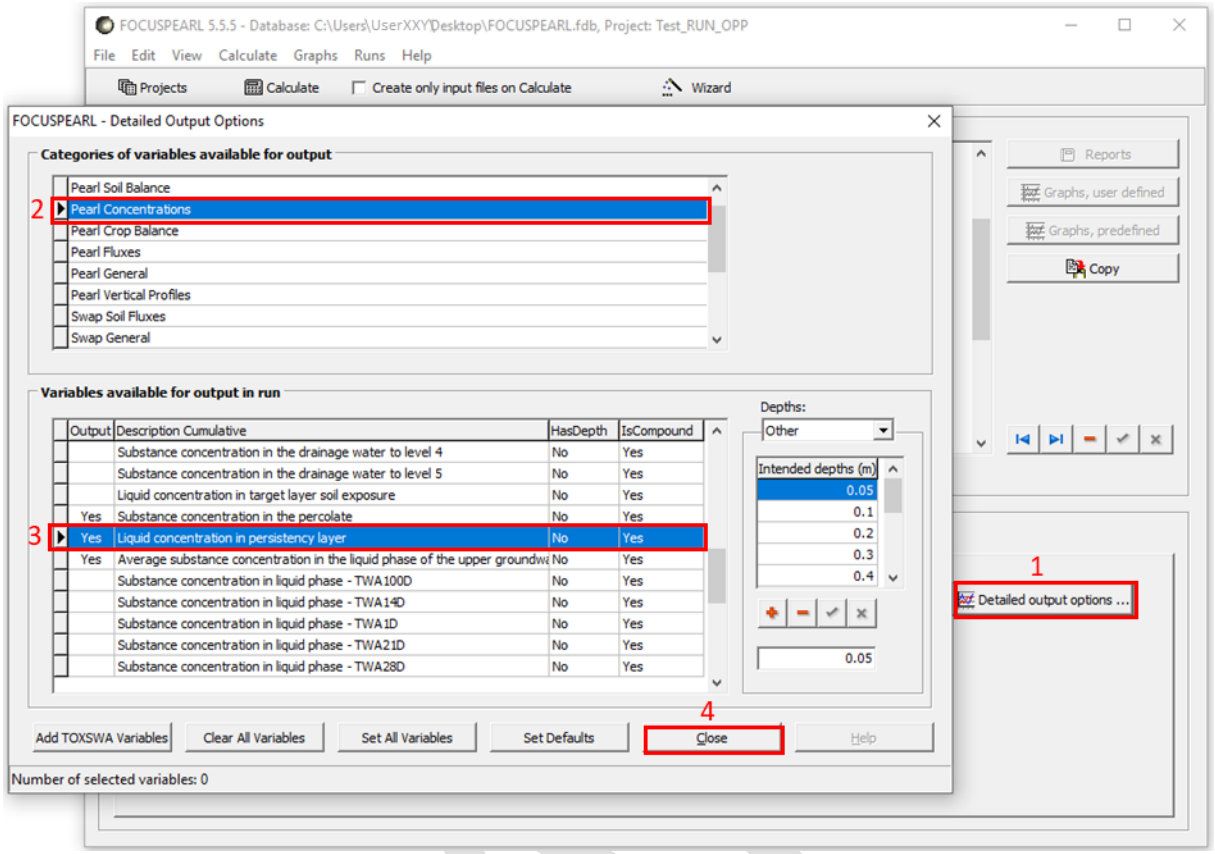
[18] According to the EFSA imidacloprid conclusion, conifers (grouped with non-orchard trees) are noted as not foraged by bees for nectar and pollen/ considered as non-attractive to bees for pollen and nectar. (https://statics.teams.cdn.office.net/evergreen-assets/safelinks/1/atp-safelinks.html)
[19] According to https://ecologyisnotadirtyword.com/2016/10/30/unlikely-plant-pollinator-relationships/ conifers are an important source of resins for some bee species, who use it to build nests and as chemical defence against predators.



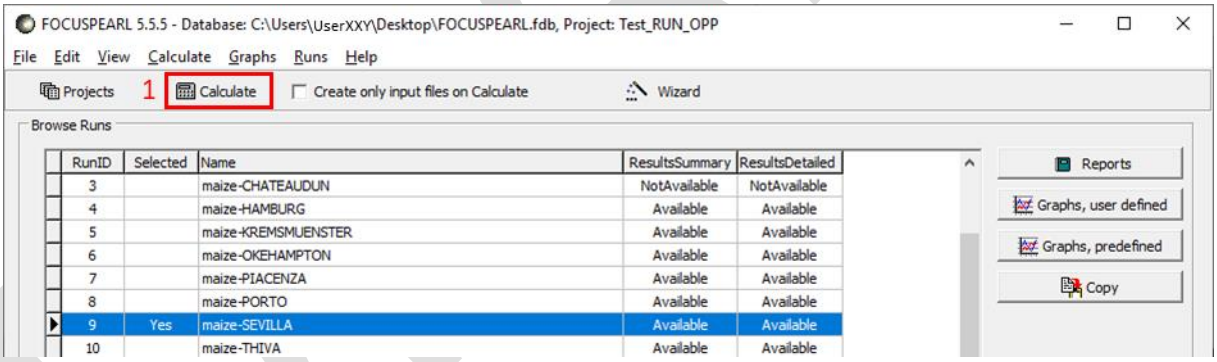
- 2 Set the print method on "Day", Set the top of the "Ecotoxicological averaging depth" to 0 m and the bottom boundary to the soil depth 0.2 m (see EFSA Guidance, Chapter 5.5.15).
  Mark the checkbox for detailed output. Click on the post edit button (the '√' button on the bar on the Browse part of the main screen)



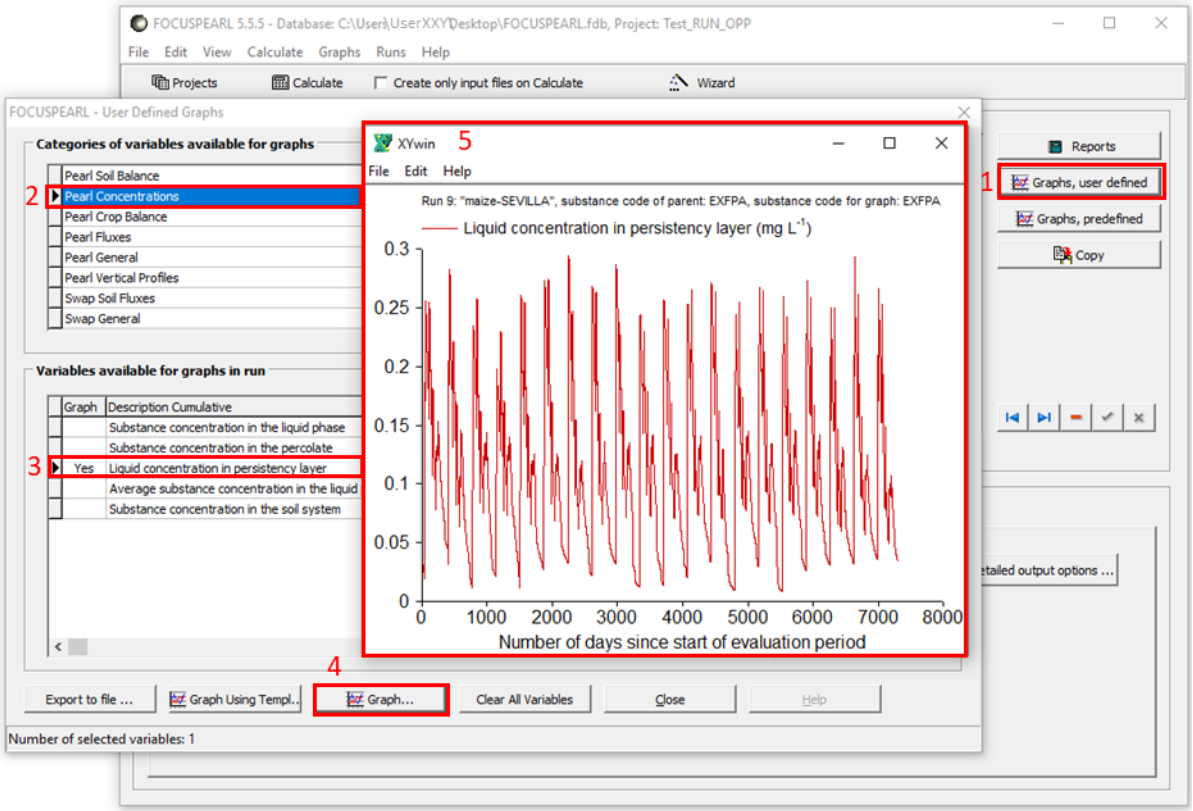
- 3 Click on the button 'Detailed output options' and select 'PEARL concentrations'. Next double click on the output item for 'Liquid concentration in persistency layer', i.e. the top 0.2 m layer.



- 4 Execute the assessment

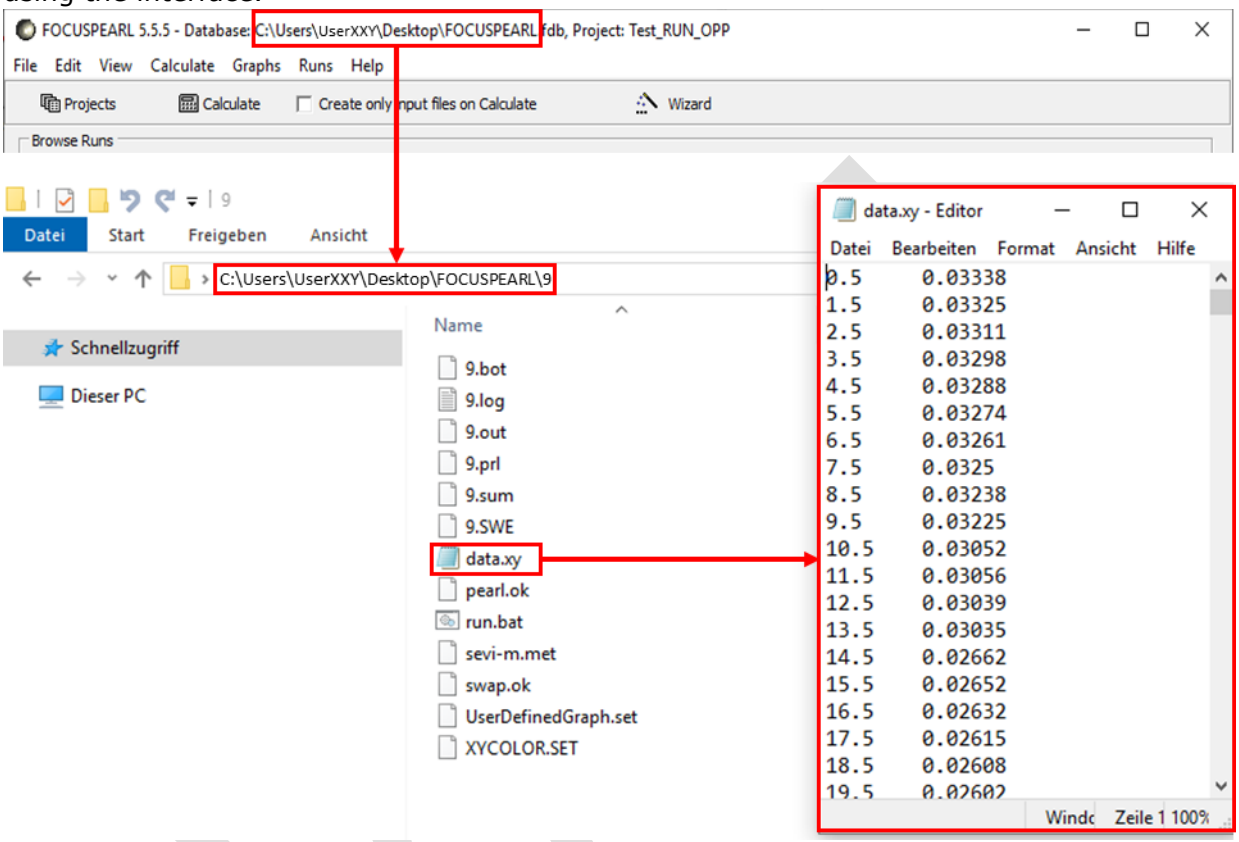


- 5 After completion of the assessment click on the button 'Graphs User Defined', select the category PEARL concentrations and double click on the item for liquid concentration in the persistency layer. Next click on the 'Graph' button. A graph will be shown with the pore water concentration in the top 20 cm layer plotted against time.


FOCUSPEARL 5.5.5 - Database: C:\Users\UserXXY\Desktop\FOCUSPEARL.fdb, Project: Test_RUN_OPP
File Edit View Calculate Graphs Runs Help
Projects
Calculate
Create only input files on Calculate
Wizard
FOCUSPEARL - User Defined Graphs
Categories of variables available for graphs
Pearl Soil Balance
Pearl Concentrations
Pearl Crop Balance
Pearl Fluxes
Pearl General
Pearl Vertical Profiles
Swap Soil Fluxes
Swap General
Variables available for graphs in run
Graph
Description Cumulative
Substance concentration in the liquid phase
Substance concentration in the percolate
Yes
Liquid concentration in persistency layer
Average substance concentration in the liquid
Substance concentration in the soil system
Export to file ...
Graph Using Templ..
Graph...
Clear All Variables
Close
Help
Number of selected variables: 1
Reports
Graphs, user defined
Graphs, predefined
Copy
1
2
3
4
5
XYwin
File Edit Help
Run 9: "maize-SEVILLA", substance code of parent: EXFPA, substance code for graph: EXFPA
Liquid concentration in persistency layer (mg L-1)
0.3
0.25
0.2
0.15
0.1
0.05
0
0 1000 2000 3000 4000 5000 6000 7000 8000
Number of days since start of evaluation period

1
2

- The data for this graph are output to the data file data.xy; this file is available in the folder [RunID] (in the example RunID 9) containing the PEARLdb.fdb file (path shown on top of the main screen). Please note that this file is overwritten if you plot another graph using the interface.



The relevant time points for bee assessment are 120 days for grassland and 150 days for arable land (see EFSA Guidance, chapter 5.5.15).

EUROPEAN CHEMICALS AGENCY
P.O. BOX 400, FI-00121 HELSINKI, FINLAND
ECHA.EUROPA.EU